NICKEL TOXICITY IN PLANTS:

THE PHYSIOLOGICAL RELATIONSHIP EXISTING BETWEEN PLANTS AND THE SERPENTINE SOILS ON WHICH THEY GROW.

By

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
EXPERIMENTAL METHODS	2
Soil Culture	3
Sand Culture	3
Solution Culture	5
Nutrient Solutions	7
ANALYTICAL METHODS	10
Plant Analysis	10
Soil Analysis	24
ADAPTION OF AN EXISTING METHOD FOR THE DETERMINATION OF NICKEL	27
SERPENTINE SOILS	39
Review of the literature	39
Examination of the Whitecairns soils	49
Effect of change in soil pH on uptake of nickel	51
THE ROLE OF NICKEL IN PLANT GROWTH	59
Effect of pH on the absorption of nickel	63
The rate of absorption of nickel by oat plants	68
CONCLUSIONS	74
IRON DEFICIENCY IN PLANTS	78
Review of the literature	78
THE NICKEL-IRON RELATIONSHIP	88
Nickel-iron ratio experiments	95
Distribution of iron in nickel-toxic oat plants	109
Further aspects of the nickel-iron relationship	119
Effect of molybdenum on nickel toxicity	127
CONCLUSIONS	132
RELATIONSHIP BETWEEN NICKEL TOXICITY AND MAJOR NUTRIENT SUPPLY	137
Effect of a low level of major nutrients	138
Effect of a high level of major nutrients	143
CONCLUSIONS	161

TABLE OF CONTENTS (contd.)

	Page
UPTAKE OF CHELATE METALS BY OAT PLANTS	162
A note on the form of nickel in the Whitecairns basin soil	162
Uptake of chelate nickel by oat plants	168
Uptake of chelate forms of manganese, copper, zinc and cobalt by oat plants	177
CONCLUSIONS	181
GENERAL CONCLUSIONS	184
SUMMARY	193
ACKNOWLEDGEMENTS	199
REFERENCES TO THE LITERATURE	200
APPENDIX	212

INTRODUCTION

Oat plants showing symptoms quite unlike those associated with any common nutrient deficiency were received at the Macaulay Institute for Soil Research during the summer of 1949, from a field at Whitecairns, about 9 miles north of Aberdeen.

The symptoms took the form of white longitudinal stripes on the leaves of affected plants, often associated with a certain amount of a diffuse or striped type of chlorosis. Similar oat plants from Ellon, Aberdeenshire, had been examined by the Spectrochemistry Department of the Institute in 1941 and had been found to contain unusually high concentrations of nickel (Mitchell (93)) as did the soils on which they grew. Spectrochemical analysis of the plants and soil from Whitecairns showed that they also contained excessive levels of nickel. The obvious inference was that the plants were being poisoned by the large amounts of nickel in the soil, although this diagnosis was not finally proved until Vergnano (132) carried out an investigation into the effects of nickel on plant growth and showed that similar symptoms could be induced in oats grown in sand-culture when nickel was supplied in the nutrient solution.

Her findings will be discussed more fully in the relevant sections of this thesis, which is devoted to the examination of certain aspects of nickel toxicity which appeared to warrant further study.

EXPERIMENTAL METHODS

In the plant nutrition studies now to be described, the choice lay between soil-, sand-, or solution culture. Sand culture was chosen in most cases in preference to soil-culture because the nutrient interrelationships were less complex. Solution culture was used in cases where a more rigorous control of substrate pH was required. The oat plant was used in the investigations except where indicated otherwise; the variety was Victory (a Swedish strain).

In the summer, soil- and sand culture experiments were carried out in a bird-proof cage, while in the winter a heated greenhouse was used, which was provided with artificial illumination from daylight fluorescent tubes arranged to supplement the natural daylight for 16 hours daily (as described by Low (76)). All solution culture work was done in the greenhouse, irrespective of season.

Containers

Large clay pots (9 in. diameter) which had been painted (inside) with two coats of bitumastic paint were used for soil- and sand-culture work. Hewitt (45) has shown that such pots are satisfactory for major-element and certain trace-element studies although not suitable for the most refined trace-element deficiency work. For solution culture, glazed porcelain crocks of 5 litre capacity were employed.

Soil Culture/

Soil Culture

Bulk samples (several cwts.) of soil were removed from the experimental area, partly air-dried, and riddled to remove roots and stones. After thorough mixing, a small sub-sample was withdrawn for analysis. The remainder of the soil was mixed with a relatively pure quartz sand in the proportion of 7 soil : 2 sand. This mixture was used as a medium for growing plants, the addition of sand ensuring free drainage conditions. Bottom drainage was provided by covering the hole at the base of the pot by a wire grid and a thin layer of $\frac{1}{4}$ in. washed granite chips. When insoluble or relatively insoluble salts had to be added they were mixed with the air-dry soil-sand mixture prior to potting. After potting, 100-120 seeds per pot were sown. After germination seedlings were thinned to 80 per pot and adequate water supplied as required.

Sand Culture

Investigations of factors influencing ion absorption call for closer control of experimental conditions than can be achieved in soil culture, and in view of its comparative simplicity and general acceptance as a medium for plant growth (Miller (87)), sand culture is widely employed.

A relatively pure quartz sand from Leighton Buzzard (13% held by a 14-mesh and 99% by a 30-mesh British Standard Sieve) was used which, on the average, contained the following amounts of nutrients (expressed as ppm. in the sand):

NO_3/

NO_3, trace; P, 0.06; K, 1.08; Ca, 23.5; Mg, 6.3; Fe, trace; Ni, 0.017. These were the amounts extracted by 0.5N acetic acid. When 50 g. sand was boiled for 10 minutes with 200 ml. 15% hydrochloric acid the concentration of iron in the solution was 13.2 ppm. The particle size of this sand, which approaches that recommended by Ellis and Swaney (25), enables satisfactory root aeration to be maintained more easily than is possible with a finer material, but its lower water-holding capacity is a disadvantage.

Before filling the pots, the sand was flushed with water, but not otherwise treated. The same type of bottom drainage was used as for soil cultures. Seeds were sown in the damp sand, covered with a further inch of sand, watered and the pot covered with a sheet of glass to maintain the necessary humidity for germination. When the shoots were $\frac{1}{4}$-$\frac{1}{2}$ in. high, 600 ml. of nutrient solution was supplied once daily for 7 days. After that time, solution was supplied twice daily (600 ml. and 400 ml.), this volume being in excess of the moisture-holding capacity of the sand in the pot. Before each addition of nutrient solution the sand was thoroughly flushed out with water to remove any nutrient solution remaining from the previous application. In very hot weather, water was also given between nutrient solution applications, so that undue concentration of the solution due to evaporation or transpiration was avoided.

Solution Culture/

Solution Culture

It was found necessary to resort to solution culture whenever the nutrient solution pH had to be maintained within a definite range; sand culture, as used here, was found to be unsatisfactory in this respect, probably because of buffering compounds which accumulated in the sand and maintained the pH of the medium between 5.5 and 6.5 irrespective of the pH of the nutrient solution applied. These buffering compounds were thought to be phosphates and examination of sand in which plants had been grown confirmed the presence of water-insoluble phosphates.

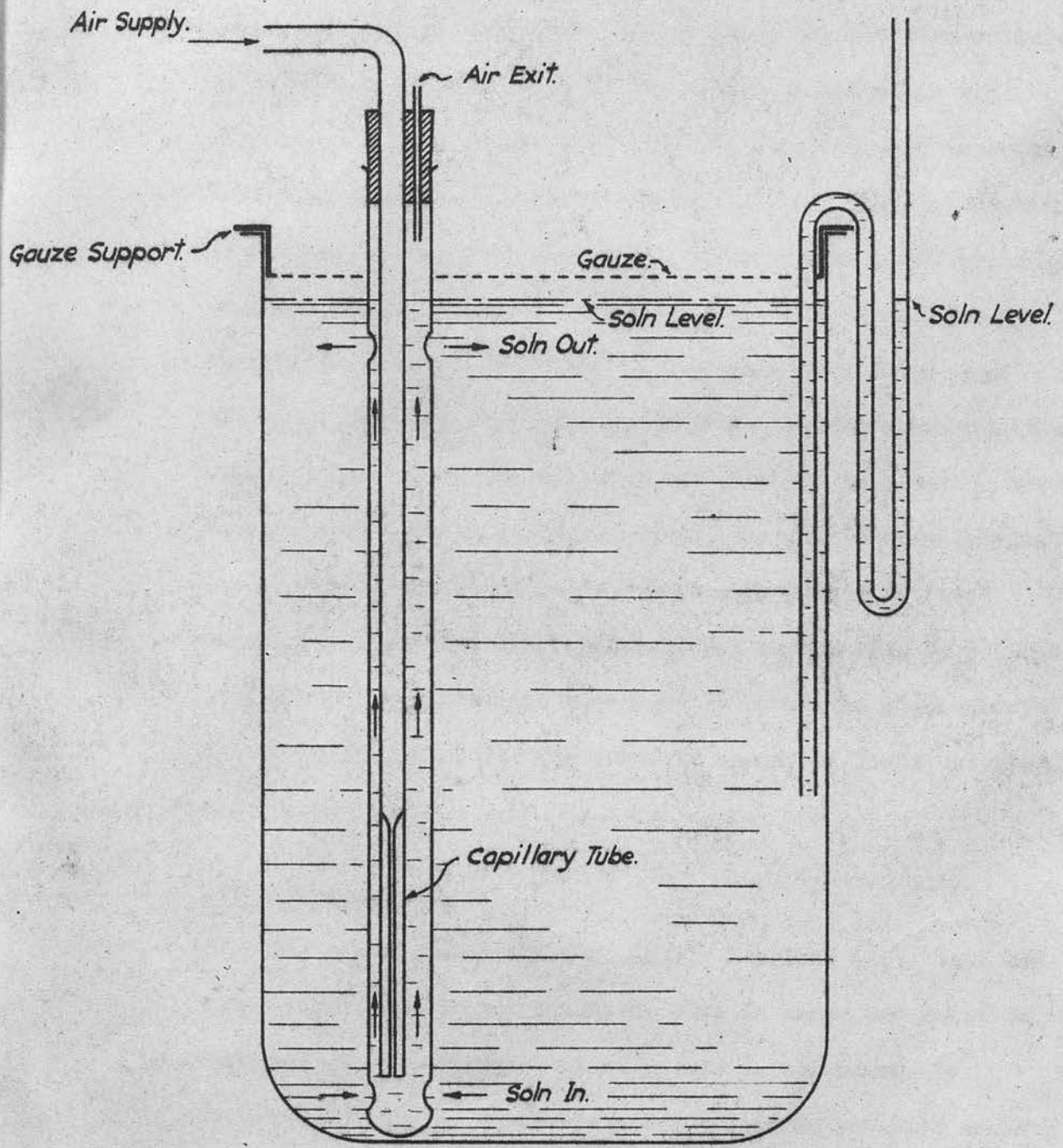
When plants are grown in solution culture, the pH of the nutrient solution changes considerably by the transfer of ions between solution and roots (Redfern, (107): Hoagland (49)) and the magnitude of the change increases as the rate of development of the plants increases. Arnon et al. (3) in examining the effect of pH on nutrient absorption overcame this difficulty by growing a small number of plants in a large volume of nutrient solution, and by adjusting the pH twice daily; in this way the pH was easily maintained within $\pm$ 0.2 pH units. In the experiments described here it was not possible to have conditions similar to those used by Arnon and his co-workers in view of the relatively large weight of plant material required for analysis. It was found, however, that for nutrient solutions of the composition employed here, and for a stand of 50 oat plants in 5 litres of solution, the pH could/

could be maintained within ± 0.25 pH units if adjusted twice or thrice daily. In this way, the effect of a pH range (say 4.0 - 4.5) was examined rather than the effect of a definite pH value.

In adjusting the pH of the solutions, a 100 ml. sample was withdrawn from the crock and its pH determined by means of a glass electrode pH meter. Dilute sulphuric acid (or sodium hydroxide) was then added to the 100 ml. until the pH was reduced to the stable end of the range under examination. The amount of acid (or alkali) required to adjust the total volume present in the crock was then calculated and added; the 100 ml. sample was also returned to the crock.

The solutions in the crocks were aerated continuously from an external supply of compressed air by means of an aerator assembly (see Diagram) which consisted of two glass tubes - an inner tube terminating in 3 in. of narrow bore capillary tubing and a wider outer tube sealed at the bottom but provided with two pairs of holes 1 and 9 in. from the lower end. Air passed into the inner tube displaced solution in the outer tube, forcing it out through the upper set of holes. Fresh solution was drawn in at the lower set and in this way, in addition to aeration, efficient circulation of the solution was achieved. The plants were supported on a waxed cotton gauze fixed to the lower side of a bitumen-painted wooden ring ($\frac{1}{4}$ in. thick) of slightly smaller diameter than the crock (7 in.). In 1953, plastic fly screen (Tygan) came on the market and was successfully used, after trial, in place of waxed cotton gauze.

Difficulties/


Air Supply.
Air Exit.
Gauze Support.
Gauze.
Soln Level.
Soln Level.
Soln Out.
Capillary Tube.
Soln In.

Difficulties arose when normal methods of germination and transfer were employed in the production of the large number of plants of equal vigour required for solution-culture experiments, and consequently the following technique was adopted. Eighty seeds were 'sown' in each ring and the ring buried to a depth of about an inch in a 9 in. clay pot containing sand which was watered normally. After 4-5 days the seeds had germinated and variation in size or vigour of plants was remarkably slight. When the plants were about $\frac{1}{2}$ in. high the ring, complete with plants, could be readily removed without damage to the roots by immersing the clay pot in water. After washing the roots in distilled water, the plants were immediately transferred to crocks containing the appropriate nutrient solution. The sand which was retained on the ring gave additional support to the plants and successfully prevented algal growth in the solutions by cutting down the amount of light entering the crocks.

Nutrient Solutions

It is recognised (Miller, (87): Hoagland (49)) that the composition of nutrient solutions used in sand- and solution culture can vary widely; plant growth is not adversely affected as long as nutrients are supplied in adequate amounts and in proportions which maintain physiological balance (Hoagland (49)) and as long as the pH of the nutrient solution is not extreme (Arnon et al. (3, 4)) and the osmotic pressure not excessive (Hoagland (49)).

The/

The solutions used here were based on some which had previously been found to give satisfactory growth of oats. The amount of solution given ensured that adequate nutrients were supplied except where specific deficiencies were being investigated. The pH of the nutrient solutions was adjusted to pH 5.5 before use by adding dilute acid or alkali, except where other pH values were required, and in sand culture the pH of the medium was never abnormal. Except for a few instances, where certain nutrients were being supplied at high level, the concentration of the solutions used was such that the osmotic pressure lay between 0.65 - 0.70 atmospheres, and where direct comparisons of solution effects were being made, the osmotic pressure differences were negligible. When studying the effect of change in cation level in the nutrient solution on the uptake of nickel, care was taken to ensure that the consequent changes in osmotic pressure were not excessive; unfortunately it was not possible to alter the concentration of a single ion in a solution. However, provided the limitations of this type of solution are recognised the results obtained can be rigorously treated.

Nutrient solutions were prepared from stock solutions, each containing a known concentration of a single salt dissolved in water. Measured volumes of these were mixed in a relatively large volume of water, dilute acid or alkali added to produce the pH required and the whole diluted to a predetermined volume (usually 10 or 45 litres). The

composition of the stock solutions is given in Table 1 of the Appendix. The volumes of stock solution required for 10 litres of the basic nutrient solution used for oats were as follows:- $NaNO_3$, 15 ml.; $Na_2HPO_4.12H_2O$, 20 ml.; K_2SO_4, 20 ml.; $Ca(NO_3)_2.4H_2O$, 15 ml.; $MgSO_4.7H_2O$, 15 ml.; $CaCl_2$, 2.5 ml. The composition (ppm.) of this nutrient solution was therefore: nitrogen, 138; phosphorus, 8.7; potassium, 81; calcium, 151; magnesium, 37. In certain experiments, other nutrient solutions were used; full details of these appear in the appropriate section of the text.

Table 1 also shows the amounts of trace elements (iron, manganese, copper and boron) which were included in the nutrient solutions. The amounts added were based on the recommendations of Miles, Templeman, and Pollard (86). These and other trace elements were also available to the plants from the sand, from the water which was not distilled, and from the reagent chemicals used in preparing the stock solutions.

ANALYTICAL METHODS

Plant Analysis

The use of plant analysis rather than soil analysis to assess nutrient status was established as a recognised technique in plant-nutrition studies by de Saussure (1804) and Liebig (1840). Between 1840 and 1920 many workers, Hellriegel (38) probably being the first, attempted with varying success to find methods of utilizing composition differences in plants to determine the nutrients available in the soil for their growth. Interest has revived recently with the introduction of rapid chemical tests on fresh tissue and Goodall and Gregory (31) have reviewed the whole subject in a recent communication.

Many satisfactory techniques are now available, among them those of Beauchamp (10), Emmert (26), Hoffer (50), Hunter (52), Lundegårdh (77), Plant et al. (106), and Thornton et al. (127). Some workers analyse the dry-matter or extracts of the dry matter, others use the expressed sap or extracts of fresh material or determine the concentration of nutrients by tests on the plant tissues themselves.

The method used here in the initial stages of this investigation is that developed recently by Hunter (53), in which a selected plant tissue (generally conducting tissue) is extracted by 2% acetic acid in a macerator, the extract being decolorised by activated carbon before filtration. The extract is then analysed for nitrate, phosphate, potassium, calcium and magnesium by rapid and relatively accurate colorimetric or

turbidimetric/

turbidimetric methods of chemical analysis. This procedure determines the extractable nutrients in the tissue and not the total nutrient content, although Hunter has shown that, except in the case of phosphorus, the fractions extracted normally approximate quite closely to the total amount present.

Latterly, however, when it was desirable to assess the total uptake of particular elements, it was found convenient to analyse the dry-matter extract for major elements, as well as for iron and nickel, for which purpose it had been prepared. In addition, the dry-matter of whole plants was used for this rather than separated plant parts as used for the fresh-tissue extract. Normally, 4 g. of dry-matter was ashed at 450° C, taken up in 20 ml. 5% hydrochloric acid, filtered and diluted to 100 ml. with distilled water.

The determination of trace elements is most satisfactorily carried out on dry-matter extracts. Colorimetric methods were used for nickel and iron, although certain nickel analyses and all other trace-element figures here reported were determined spectrographically in the ash of the dry-matter (Mitchell (94)). Again, it was found convenient to determine calcium and potassium together using flame photometry, rather than by the separate turbidimetric methods developed by Hunter (54, 55).

Before describing the methods in detail, the following brief notes will indicate the general principles underlying the methods used, and explain the technique of using a calibration graph (for each method) prepared from standard solutions to calculate the amounts of each element in 'unknown' solutions.

Nitrogen/

Nitrogen: Kjeldahl digestion followed by a modified Conway micro-diffusion technique.

Nitrate: Colorimetric phenoldisulphonic acid.

Phosphorus: Colorimetric molybdenum blue using hydrazine sulphate.

Potassium: Flame photometer (Filters, image convertor, photo-multiplier).

Calcium: Flame photometer (Monochromator, photo-multiplier).

Magnesium: Colorimetric thiazol yellow, using excess of dye reagent which is then determined and the magnesium value in the solution obtained by difference.

Iron: Colorimetric α-α' dipyridyl.

Nickel: Colorimetric dimethylglyoxime.

Calibration Graphs

Standard solutions of appropriate concentration are prepared for use with each method. A range of volumes of the standard solution (usually 0, 2, 4, 6, 8 and 10 ml.) is treated exactly as described in the sections below and the resultant colour intensities measured using a Spekker photo-electric absorptiometer. A graph is then constructed in which Spekker reading is plotted against known concentration of the element. A fresh graph is prepared with each batch of analyses and from the Spekker readings given by an appropriate aliquot of the plant or soil extract, the concentration of the element in question can be calculated./

calculated. The table below gives details of amounts of standard solutions, size of Spekker cells, and filters used in each determination.

TABLE 1

Details of Calibration Graphs etc.

Method	Volume of standard solution used	Spekker details: Size of cell	Spekker details: No. and type of filter
Ammonium	0,2,4,6,8,10 ml. (diluted to 10 ml. with Morgan's reagent).	4 cm.	No. 604 (gelatine) Green.
Nitrate	0,0.5,1.0,1.5,2,3 and 4 ml. (Diluted to 4 ml. with water).	4 cm.	No. 7 (glass) Violet.
Phosphate	0,2,4,6,8,10 ml. (diluted to 100 ml. with water).	1 cm.	No. 1 (glass) Red.
Magnesium	0,2,4,6,8,10 ml. (diluted to 10 ml. with Morgan's reagent and 5 ml. calcium compensating reagent added.)	4 cm.	No. 7 (glass) Violet.
Iron	0,2,4,6,8,10 ml. (diluted to 10 ml. with water).	4 cm.*	No. 604 Spectrum Green (gelatine)
Nickel	0,2,4,6,8 ml. (diluted to 8 ml. with water).	4 cm.	No. 601 Spectrum Violet (gelatine)

* or on 10 cm. cell E. E. L. Absorptiometer using No. 604 green filter.

Determination/

Determination of Total Nitrogen

0.25 g. dry-matter is digested in a 100 ml. Kjeldahl flask with 10 ml. concentrated sulphuric acid, 1.5 g. potassium sulphate and a pinch of copper sulphate. The flask is heated fairly vigorously, excessive frothing and loss of acid being avoided. The heating is continued until the digest is colourless or pale yellow, when the flask is allowed to cool, 5 - 10 ml. water added and the contents of the flask boiled for about 45 minutes. The digest is allowed to cool, when it is transferred to a 250 ml. standard flask and made up to volume. The concentration of ammonium ions in the digest is determined by means of a diffusion technique followed by colour development with Nessler's reagent as described below, the digest being made alkaline with 5 ml. 15% sodium hydroxide instead of potassium carbonate solution.

Determination of Ammonium by Diffusion Technique

Reagents

(1) Morgan's Reagent: 100 g. hydrated sodium acetate (analytical reagent) dissolved in water, 30 ml. glacial acetic acid (analytical reagent) added and the mixture made up to 1 litre with water.

(2) Nessler's Stock Reagent: 68 g. potassium iodide (analytical reagent) and 27 g. mercuric chloride dissolved in about 250 ml. water and added to a solution of 112 g. potassium hydroxide/

hydroxide (analytical reagent) dissolved in about 250 ml. water. The mixture of the combined solutions is made up to 1 litre, mixed, and allowed to stand for several days. Aliquots of the supernatant liquid can then be removed as required. The reagent should be stored in the dark.

(3) Nessler's Diluted Reagent: Nessler's stock reagent diluted with four times its volume of water. This should be prepared when required.

(4) 0.5% Sulphuric Acid: 5 ml. concentrated sulphuric acid (analytical reagent) added to 1 litre water and mixed.

(5) Ammonium Stock Solution: 500 ppm. ammonium. 1.832 g. ammonium sulphate (analytical reagent) dissolved in Morgan's reagent and diluted to 1 litre with Morgan's reagent.

(6) Ammonium Standard Solution: 25 ppm. ammonium. 50 ml. ammonium stock solution diluted to 1 litre with Morgan's reagent.

(7) Potassium Carbonate Reagent: 500 g. anhydrous potassium carbonate (analytical reagent) dissolved in 500 ml. water and filtered (Whatman No. 531) if necessary.

Procedure

An aliquot of the extract containing 0.025 - 0.250 mg. ammonium is placed in a tall 30 ml. beaker and made up to 10 ml. with Morgan's reagent. After these operations no liquid should be adhering to the sides of the beaker above the surface of the solution.

A/

A folded 7 cm. filter paper (Whatman No. 1) is placed in a filter funnel and filled twice with 0.5% sulphuric acid; when completely drained, the paper is removed and excess moisture wiped off on a clean filter paper.

The aliquot of the extract, made up to volume as described above, is then made alkaline by the addition of 2 ml. potassium carbonate reagent and the solution mixed by gentle rotation. The soaked filter paper is placed loosely in the mouth of the beaker which is then placed on a glass plate and covered with a large beaker. All operations in this paragraph should be carried out as rapidly as possible.

The apparatus is left overnight (preferably two nights) during which time the ammonia diffuses from the mixture to the filter paper. The filter paper is transferred to a funnel, very thoroughly washed with water and the washings collected in a 50 ml. standard flask. The contents of the flask are made alkaline with 1 ml. 15% sodium hydroxide, mixed, and 10 ml. Nessler's diluted reagent added and mixed.

The intensity of the brown colour is determined within 2 hours of adding the Nessler's reagent.

Source of Method

The method is a modification of that by Conway, E.J. and Byrne, A.; Biochem. J., (1933), 27, 419. The Nessler's stock reagent is a modification of that described by Vanselow, A.P.; Ind. Eng. Chem. (Anal.), (1940), 12, 516.

Determination of Nitrate

Reagents

(1) Phenoldisulphonic Acid: Purchased as 20% solution in concentrated sulphuric acid. When a white precipitate is present it can be removed by slightly raising the temperature.

(2) Nitrate Stock Solution: 2000 ppm. nitrate. 2.742 g. sodium nitrate (analytical reagent) dissolved in water and diluted to 1 litre.

(3) Nitrate Standard Solution: 100 ppm. nitrate. 50 ml. nitrate stock solution diluted to 1 litre with water.

Procedure

A suitable aliquot (which should contain 0.05 - 0.40 mg. nitrate) is put in a 100 ml. conical flask and 0.5 ml. 15% sodium hydroxide added per ml. of undiluted extract. 1 ml. 20 vol. hydrogen peroxide (analytical reagent) is then added and the whole evaporated slowly to dryness. Successive additions and evaporations of hydrogen peroxide should be continued, the flask being cooled before each addition, until a white residue is obtained. 1 ml. water is then added and evaporated to dryness. After cooling, 4 ml. water are added and mixed until the residue is dissolved, warming if necessary. 4 ml. phenoldisulphonic acid are then added, swirling all the time and bringing any nitrate which is on the side of the flask into contact with the liquid. After about 10 to 15 minutes, 30 ml. water are added and mixed and then 40 ml. 15% sodium hydroxide/

hydroxide are added. The mixture is allowed to cool to at least 20° C and the intensity of the lemon colour determined.

Source of Method

The method differs in detail only from the usual phenoldisulphonic technique.

Determination of Phosphate

Reagents

(1) Indicator: 0.1 g. β-2:6 dinitrophenol dissolved in a mixture of 25 ml. rectified industrial spirits and 75 ml. water.

(2) Molybdate Reagent: 10 g. sodium molybdate dissolved in 1 litre of distilled water containing 100 ml. of concentrated sulphuric acid.

(3) Hydrazine Reagent: 10 g. hydrazine sulphate dissolved in 1 litre water.

(4) Phosphate Stock Solution: 600 ppm. phosphorus. 6.928 g. sodium phosphate (analytical reagent) dissolved in water and diluted to 1 litre.

(5) Phosphate Standard Solution: 30 ppm. phosphorus. 50 ml. of phosphate stock solution diluted to 1 litre with water.

Procedure

If the acidity or alkalinity of an aliquot is excessive, it must be adjusted before the phosphate content is determined.

The/

The aliquot (containing 0.03 - 0.30 mg. phosphorus) is diluted with 50 ml. water in a 250 ml. conical flask and 2 drops of indicator are added. If the contents of the flask are colourless, 15% sodium hydroxide is added from a burette until the mixture is slightly alkaline (pale yellow) and then dilute sulphuric acid is added until the mixture is slightly acid (colourless). If on adding the indicator the mixture is yellow, dilute sulphuric acid is added until the yellow colour is discharged. The contents of the flask are then diluted to 100 ml. with water, and 5 ml. molybdate reagent are added, followed immediately by 5 ml. hydrazine reagent. The contents of the flask are mixed and boiled for 1 minute, evaporation being kept at a minimum and constant amount. The solution is allowed to cool and the intensity of the blue colour determined.

Source of Method

The method is derived from Shelton and Harper; Iowa State College J. of Sci.; (1941), 15, 403.

Determination of Potassium and Calcium

The concentration of these elements in the fresh-tissue or dry-matter extract was determined by means of a flame photometer.

Determination of Magnesium

Reagents

(1) Oxalic Acid Reagent: 15 g. hydrated oxalic acid dissolved in water and diluted to 1 litre.

(2) Tartrate Reagent: 10 g. sodium hydrogen tartrate, 10 g. mannitol and 2.5 g. hydrazine sulphate dissolved separately in water, and the mixed solutions diluted to 1 litre.

(3) Dye Reagent: The concentration of dye required will depend on the particular specimen of dye used and should be chosen to give a convenient range of intensity readings. The following may prove satisfactory:- 0.09 g. Thiazol Yellow (General Analine Works, Rensselaer, New York) or 0.15 g. Titan Yellow (British Drug Houses) dissolved in water, diluted to 1 litre, mixed with two drops of 15% sodium hydroxide, and filtered (Whatman No. 1) after 24 hours. The reagent is stable for several months. Thiazol Yellow is the most satisfactory.

(4) Dye Solvent: 600 ml. butan-1-ol (pure technical normal-butyl alcohol) mixed with 400 ml. ethanol (rectified industrial spirits).

(5) Magnesium Stock Solution: 400 ppm. magnesium. 4.054 g. magnesium sulphate (analytical reagent) dissolved in Morgan's reagent and diluted to 1 litre with Morgan's reagent.

(6) Magnesium Standard Solution: 20 ppm. magnesium. 50 ml. magnesium stock solution diluted to 1 litre with Morgan's reagent.

(7)/

(7) Calcium Compensating Stock Reagent: 4000 ppm. calcium. 10 g. calcium carbonate (analytical reagent) added to about 100 ml. water in a covered beaker and about 10 ml. glacial acetic acid added. The mixture is warmed and about 500 ml. Morgan's reagent added and warmed until the carbonate has dissolved. The solution is cooled and made up to 1 litre with Morgan's reagent.

(8) Calcium Compensating Reagent: 200 ppm. calcium. 50 ml. of calcium stock solution diluted to 1 litre with Morgan's reagent.

Procedure

An aliquot of the extract (containing 0.02 - 0.20 mg. magnesium) is transferred to a 100 ml. conical flask and made up to 15 ml. with Morgan's reagent. If the aliquot is more than 5 ml. it must be evaporated to dryness and the residue dissolved in 15 ml. Morgan's reagent.

5 ml. oxalic acid reagent are added to the solution, which is mixed and left for at least one hour at room temperature.

5 ml. of tartrate reagent are then added, followed in succession, by exactly 5 ml. of dye reagent and 20 ml. of 15% sodium hydroxide. The contents of the flask are mixed after each addition and the final mixture left overnight.

50 ml. of the dye solvent are then added and shaken with the mixture for about 30 seconds.

After/

After standing for 1 minute, two phases will have separated. Within 30 minutes as much of the upper layer as can be conveniently decanted off is transferred to a small conical flask containing 0.5 ml. acetone. The contents of this flask are then mixed, and the colour intensity of this orange solution determined within 30 minutes.

Source of Method

Hunter, J.G.: Analyst; (1950), 75, 91-99.

Determination of Iron

Reagents

(1) Dipyridyl Reagent: 0.1% dipyridyl in 0.1N hydrochloric acid.

(2) Hydroxylamine hydrochloride Reagent: 10% hydroxylamine hydrochloride in distilled water.

(3) Buffer Solution (pH 4.6): To 3 litres distilled water add 400 ml. glacial acetic acid (analytical reagent) and 1150 ml. 4N sodium hydroxide. Mix well and adjust pH to 4.6 by adding either acetic acid or caustic soda.

(4) Iron Stock Solution: 100 ppm. iron.
0.8640g. ferric alum dissolved in water and made up to 1 litre.

(5) Iron Standard Solution: 5 ppm. iron.
50 ml. iron stock solution diluted to 1 litre.

Procedure/

Procedure

Pipette a suitable aliquot (which should contain between 10 and 50 γ iron) into a 100 ml. standard flask. Add 25 ml. buffer solution and 10 ml. dipyridyl reagent and adjust pH to 4.6. Add 2 ml. hydroxylamine hydrochloride reagent and allow colour to develop for at least one hour, before measurement.

Source of Method

Nicholas D.J.D., (1951) Mimeo publication Long Ashton Res. Sta., Bristol.

Determination of Nickel

Reagents

(1) Dimethylglyoxime reagent: Dissolve 0.5 g. dimethylglyoxime in 500 ml. 50% ammonium hydroxide and add 500 ml. 10% citric acid solution.

(2) Iodine Solution: Dissolve 24 - 24.5 g. iodine in a solution of potassium iodide containing 30 - 35 g. of the salt and make up to 1 litre.

(3) Nickel Stock Solution: 1000 ppm. nickel. Dissolve 0.4479 g. nickel sulphate in water and make up to 1 litre.

(4) Nickel Standard Solution: 12.5 ppm. nickel. Dilute 12.5 ml. nickel stock solution to 1 litre.

Procedure/

Procedure

Pipette a suitable aliquot (which should contain between 0.025 and 0.075 mg. nickel) into a 100 ml. standard flask. Dilute with water to about 50 ml. and add 1 ml. of the iodine solution, followed by 10 ml. of the dimethylglyoxime reagent. Dilute to 100 ml. and allow colour to develop for at least 40 minutes before measurement.

Source of Method

High, J.H. (1945), Analyst, 70, 258-9.

Soil Analysis

Sampling

The soil samples were taken using a 1 in. diameter soil auger. Each sample weighed about 2 lb. and was made up of cores taken at random from the area being sampled. The sample was air-dried at 25° C and sieved through a 2 mm. round-holed sieve, the residue retained by the sieve being further treated with a wooden rolling pin to break down the soil particles. This process was continued until no more of the sample would pass through the sieve; the residue was discarded and the material which had passed through was thoroughly mixed and used for analysis.

pH/

pH

A soil-water ratio of 1:2.5 was used in determining soil pH. 10 g. of air-dry soil were shaken with 25 ml. water for at least 30 minutes and the pH of the suspension then determined using a pH meter with glass-calomel electrodes.

Loss-on-ignition

This gave a convenient, if not too accurate - measurement of the organic matter content of the soil. A known weight of soil (oven-dried at 105° C) was heated to bright redness for 1 hour, the percentage loss in weight being the loss-on-ignition (Piper (105)).

Readily soluble phosphate, potassium, calcium, magnesium and nickel.

20 g. air-dried soil were shaken for 2 hours (major elements) or overnight (nickel) in an end-over-end shaker with 800 ml. 0.5N acetic acid and filtered (Mitchell (94)). Analyses were made on the filtrate as follows:-

Phosphate - Colorimetric estimation of the blue complex formed by reducing phosphomolybdic acid in acid solution by stannous chloride (Williams and Stewart (145)).

Potassium, Calcium, Magnesium and Nickel -

As described under Plant Analysis.

Exchangeable/

Exchangeable Bases

The method used was that of Piper (105) (p. 171). 20 g. air-dried soil were stirred with 200 ml. neutral N-ammonium acetate solution, left overnight and filtered. The residue was leached with more ammonium acetate solution until one litre of filtrate was obtained. This was evaporated to dryness and organic matter removed by oxidation with hydrogen peroxide. The residue was dissolved in 1:4 hydrochloric acid and diluted to 100 ml. with water. The individual cations in this solution were determined as describedabove.

Expression of results

Plant analysis: Major elements as parts per million (ppm.) in fresh-tissue extracts or per cent in the dry-matter. Trace elements as parts per million (ppm.) in the dry-matter.

Soil Analysis: Major elements as mg. per 100 g. of soil (readily soluble nutrients) or milli-equivalents per 100 g. soil (exchangeable bases). Trace elements as parts per million (ppm.) in the soil.

All results are on an air-dry basis.

THE DETERMINATION OF NICKEL IN PLANTS AND SOILS

Nickel determinations reported in Vergnano's thesis were made spectrographically (Mitchell (94)). The standardised technique developed in the Department of Spectrochemistry in the Macaulay Institute for the complete trace-element determination of a sample proves uneconomical when applied to the determination of a single constituent, such as nickel. It was therefore decided to consider the possibility of determining nickel in plant dry-matter and soil extracts by chemical means. A check on possible reagents soon showed that a colorimetric, rather than gravimetric method would be more suitable, both from speed and amount of nickel likely to be found in each sample. Of the possible reagents, dimethylglyoxime appeared to offer the best prospects of success. Several colorimetric methods involving its use were selected from the literature. These were mainly designed to measure nickel in the presence of large amounts of iron or copper as, for example, in metallurgical practice. In general, they involved the development of a red colour in alkaline solution, a suitable oxidising agent having previously been added to oxidise the nickel to the higher valency state. Bromine water was the most common oxidising agent used, but the use of an iodine solution, as proposed by High (48), seemed likely to offer less hazard than the use of bromine water.

There/

There were possible snags, due to interfering ions, and of these, iron, manganese and magnesium were the most likely offenders. The general practice appeared to be to add an organic acid e.g. citric acid, to complex these ions and so prevent their interferance.

The range of nickel values likely to be encountered in plant material or soil extracts was next considered. Vergnano's analytical results gave values from 6 - 300 ppm. in the dry-matter of oat plants and 60 - 400 ppm. in the soil extracts.

Assuming a maximum aliquot of 25 ml. it was found that the amounts of reagents used in High's method would prove suitable. A standard graph using lower nickel concentrations was needed and after a few trials, that adopted was produced by using 2, 4, 6, and 8 ml. of a 12.5 ppm. standard nickel solution and reading the resultant colour on a Spekker photoelectric absorptiometer using 4 cm. cells and Ilford 601 Spectrum violet filters. These filters are most sensitive to changes in the nickel colour and gave a straight line calibration graph.

Reagents

The method developed by High was designed for the estimation of nickel in copper-base alloys where some means of controlling interference from copper was required. The dimethylglyoxime was dissolved in 50% ammonia solution and to this/

this was added an equal volume of 10% citric acid to complex the copper. High investigated the optimum amounts of iodine required, and found that 24 mg. used in conjunction with 5 ml. 0.1% dimethylglyoxime gave the best results. With less, colour development was retarded and often never reached a stable maximum; with more, the nickel colour faded too rapidly.

Nickel determination in plant material

Four samples of nickel-toxic material were ashed and taken up in 5% hydrochloric acid as described in the previous section. Nickel was determined by the colorimetric dimethylglyoxime method and the results compared with those already obtained by spectrographic means on an earlier ashing.

	Nickel in dry matter (ppm.)	
Sample	Colorimetric	Spectrographic
51/43/a/L	37	35
51/43/E/L	29	28
51/91	18	99
51/92	37	37

The agreement was excellent in three out of the four samples, and in that case the results varied so widely as to suggest that only part of the nickel had been brought into solution after ashing. In general, however, the results were encouraging and a further series of samples (on which nickel analyses had already been carried out spectrographically) was ashed and the nickel determined colorimetrically.

T A B L E/

TABLE 2

Comparison of Colorimetric and Spectrographic results on plant material

Treatment No.	Nickel in plant dry matter (ppm.) Colorimetric	Spectrographic
322	85	82
328	85	101
329	85	101
331	85	112
319	88	93
330	100	115
332	110	129
334	110	93
327	118	115
320	150	123

Again, since the results were obtained from different ashings, no direct comparison between methods is possible. The spectrographic analyses achieve an accuracy of at least ± 10% (Mitchell (94)) which is generally considered adequate in soil and plant work where experimental errors in the field are often much larger than this. Bearing in mind the use to which the analytical data would be put, it was decided that, for plant material at least, the colorimetric method offered a rapid means of assessing the level of nickel in toxic material.

Nickel/

Nickel determination in soil extracts

It would be necessary to determine nickel in soils extracted by (a) 0.5N. acetic acid - the extractant normally used to assess the level of "available" nutrients and (b) neutral N-ammonium acetate - for nutrients in "exchangeable" form.

A feature of such extracts is their colour which may vary from a very pale straw colour to a very dark orange-yellow. The colour is often a measure of the level of organic matter in the soil, being darkest when organic matter is high and vice versa although this is by no means always the case. Some of the colour may also be due to iron compounds present in the extract. Organic matter in soil extracts often interferes with chemical determinations (e.g. that of magnesium (Hunter (54)) and the general procedure followed is to destroy the organic matter by the use of successive small volumes of 20 vol. hydrogen peroxide.

It was decided to see whether the colour of the soil extracts or the presence of organic matter would have any effect on the colorimetric nickel method. This could most conveniently by done by adding a fixed amount of a soil extract (5 ml.) to known volumes of nickel standard solution, developing the colour and noting any displacement of the standard graph produced. An acetic-acid extract of a granitic soil known to be low in nickel was selected. The soil, of pH 4.2, had a loss-on-ignition of 34% and had yielded a dark yellow acetic-acid extract.

It/

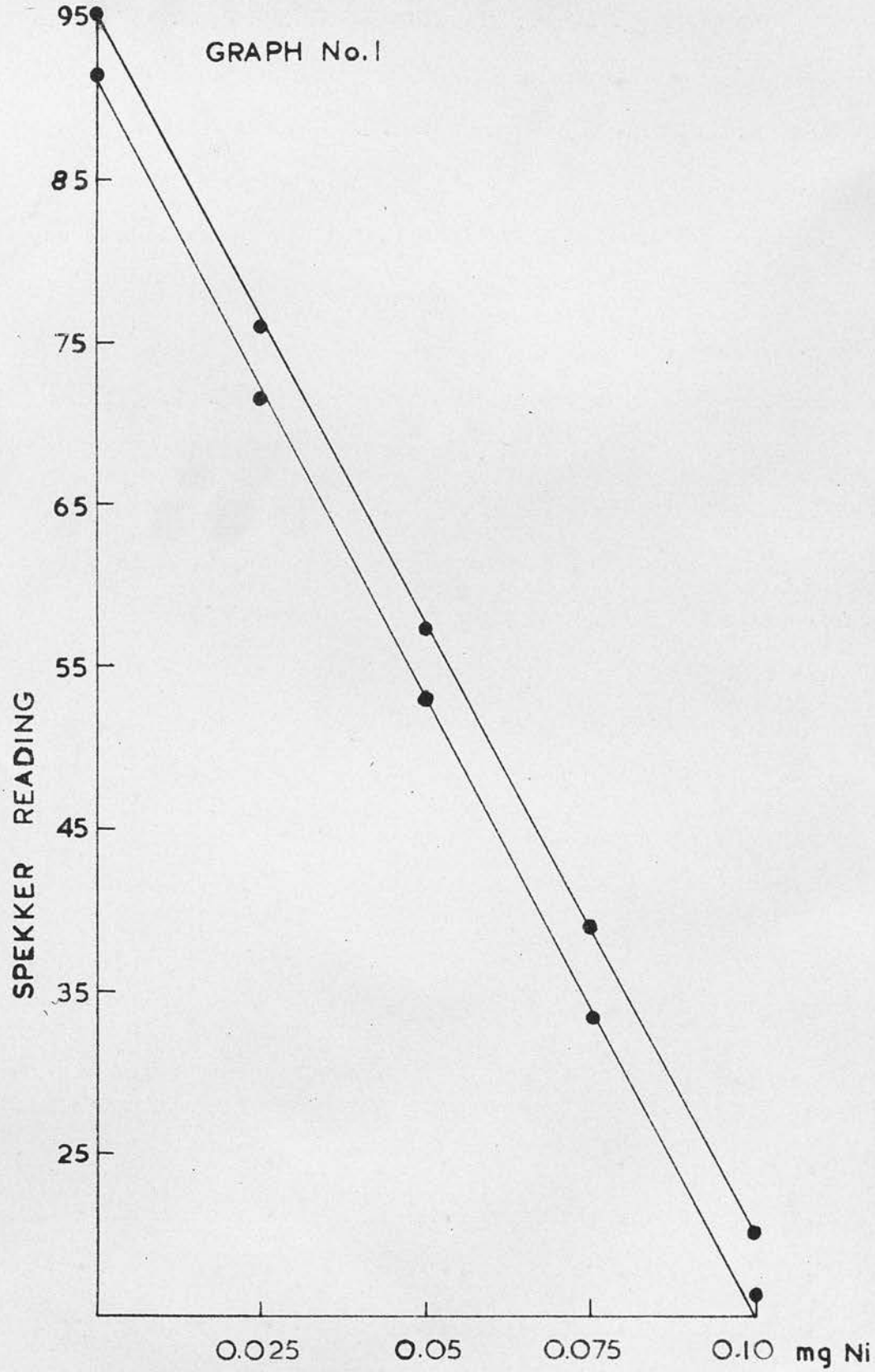
It was found that results were about 12% high as a result of adding the 5 ml. aliquot of soil extract. Further experiments were carried out in which hydrogen peroxide treatment was given to both sets i.e. the controls containing no added soil extract and the set with 5 ml. of soil extract added. The effect on the graph in the first experiment had been to displace it by a constant amount over the range of nickel values investigated so that both graphs remained parallel. (see Graph No. 1). In the second experiment the resultant graphs fell between the first two, all four still being parallel (see Graph No. 2). The results would still be high - those containing soil extract by some 5%, the controls by about 2%. The final position assumed by the graph resulting from peroxide treatment of nickel solutions to which soil extract had been added, suggested a release of organic or organically-bound nickel in small amount from the 5 ml. aliquot of soil extract used. The table below sets out these findings in terms of Spekker readings.

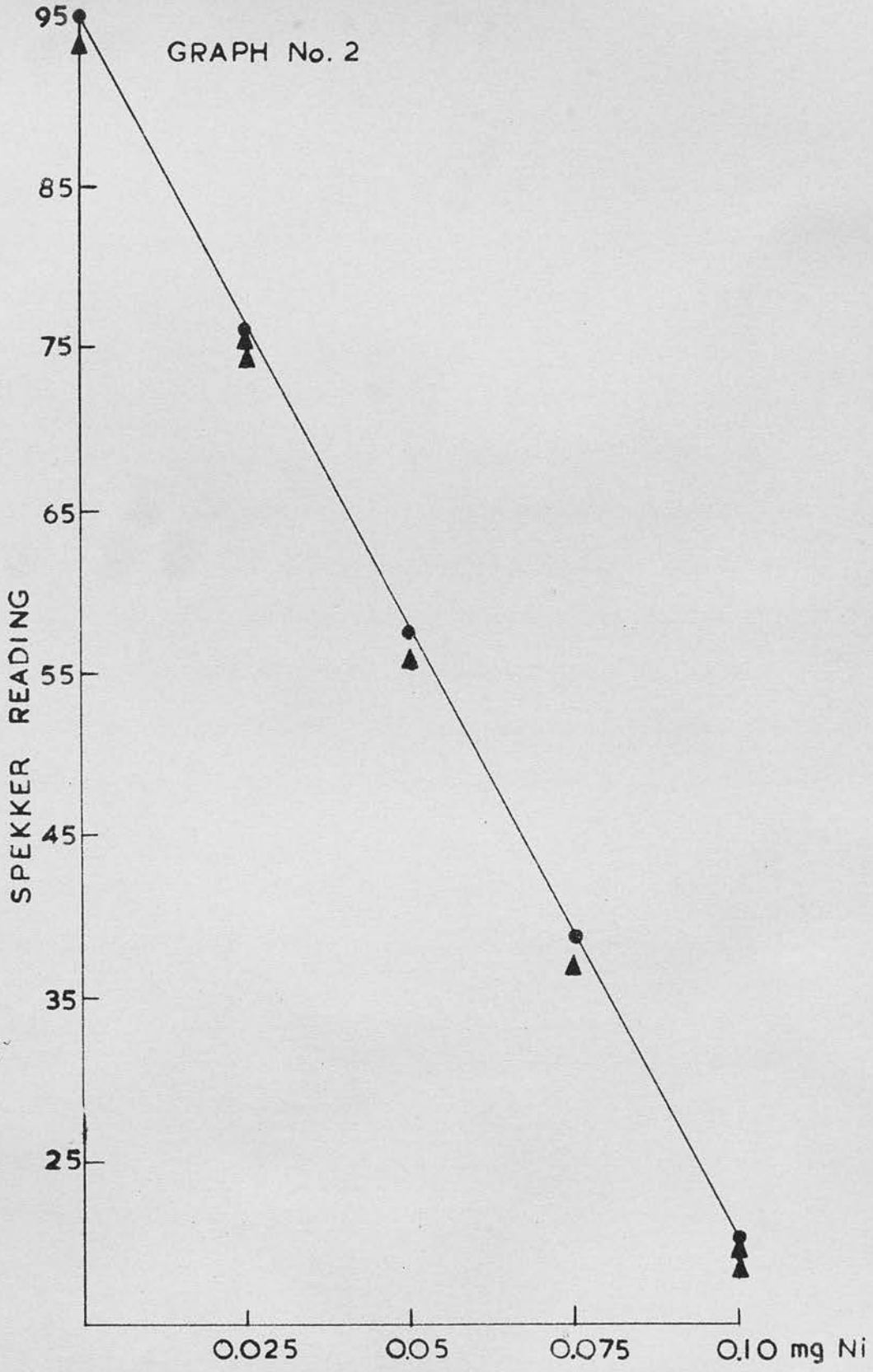
T A B L E 3

Effect of H_2O_2 on interference due to soil organic matter

Weight of nickel used (mg.)	Spekker readings			
	Untreated		Peroxide treated	
	Control	+ soil extract	Control	+ soil extract
0	95.4	91.7	93.4	93.6
0.025	76.0	71.6	74.2	75.6
0.05	57.2	53.0	56.0	55.3
0.075	38.8	33.4	37.1	36.9
0.10	20.3	16.5	18.7	19.9

Next/


GRAPH No.1
95
85
75
65
55
45
35
25
SPEKKER READING
0.025
0.05
0.075
0.10
mg Ni


GRAPH No. 2
95
85
75
65
55
45
35
25
SPEKKER READING
0.025
0.05
0.075
0.10 mg Ni

Next the effect of soil extract on colour development in the absence of nickel was investigated. Six soils, with losses-on-ignition varying from 10 - 50% were extracted overnight with 0.5 N acetic acid. 10 ml. aliquots were treated with hydrogen peroxide to destroy organic matter. The residues were dissolved in distilled water and evaporated to dryness before being taken up in 10 ml. of 5% hydrochloric acid. These samples together with similar sets of untreated 10 ml. aliquots of the soil extracts were then carried through the colour development procedure. A water blank was run as control. Finally, in order to measure the effect due to colour alone, 10 ml. soil extract aliquots were carried through the entire procedure but no dimethylglyoxime reagent was added. After forty minutes to allow for full colour development the absorption was measured by means of a Spekker photoelectric absorptiometer.

TABLE 4

Effect of soil organic matter on colour development in absence of nickel

Soil Number	Extract Colour	Spekker readings		
		Blank	Untreated	H_2O_2 treated
Water blank	-	93.8	94.2	94.0
22175	6	88.8	91.7	92.3
22338	5	88.0	89.8	93.2
22180	4	89.8	91.8	93.0
22181	3	91.4	93.0	92.6
21927	2	91.2	92.5	91.2
22007	1	90.0	93.2	93.9

6 > 1

The soils have been arranged in the table in order of depth of colour (assessed visually) of acetic-acid extract i.e. Soil 22175 gave the darkest extract, and 22007 the least coloured. It will be noted that the reduction in Spekker readings in the absence of dimethylglyoxime followed this order closely. The peroxide treatment in each case gave a Spekker reading which agreed fairly closely with that for the treated water blank. For soils low in nickel and having high organic matter contents, necessitating an aliquot of perhaps 25 ml., the results would perhaps be less near the truth.

As a test of the efficacy of the peroxide treatment, samples of the basin soil from Whitecairns which had been used in a liming experiment (described in a later section) were extracted overnight with 0.5 N. acetic acid. The basin soil has a loss-on-ignition of about 50% and yields a highly coloured extract. Nickel was determined in the extracts using the colorimetric method and the results compared with those obtained spectrographically. It should be pointed out that such acetic-soluble figures are not expected to reflect variation in the solubility of soil nickel produced by liming, since the pH of the extractant more than offsets the pH change produced by liming and so, in effect, the figures represent nickel soluble at the extractant pH. Such differences as there are do not show up any methodical differences, and so must be assumed to be a measure of sample and analytical variability.

When a similar comparison was made using ammonium acetate extracts, the agreement between results obtained by the two methods was also good.

TABLE 5

Comparison of colorimetric and spectrographic results on soil extracts

Treatment	Nickel in soil (ppm.) Color.	Spectro.	Treatment	Color.	Spectro.
626	240	240	631	239	235
627	253	265	632	235	235
628	233	215	633	239	230
629	226	240	634	228	245
630	229	230	635	215	195

Interference from other ions.

Other ions besides nickel, give colours with dimethylglyoxime. Amongst these which would normally occur in plant material and soil extracts at levels likely to interfere are magnesium, iron, manganese and perhaps copper. This much is known, but the question posed was rather different, and involved a decision regarding the amount of citric acid added per estimation to complex such interfering ions. Would 10 ml. of 10% citric acid generally suffice? Plants from, and extracts of, serpentine soils would contain much more magnesium than normal and manganese might also be present at levels higher than normal. From the plant analyses available in Vergnano's thesis (132) the maximum amounts found of these and other possible interfering ions were readily available. In the first instance the effect of each ion was investigated singly by preparing solutions containing nickel (and the ion) in the same ratio as found in plant dry matter having a nickel content of 50, 100 or 200 ppm.

It/

It was assumed that 5 g. of the dry-matter was ashed and made up to 100 ml. and that the concentration of the interfering ions in the dry-matter extracts would be constant but that the nickel varied as indicated above. Thus if the weight of nickel withdrawn per aliquot is fixed at 0.05 mg. (which corresponds to the amount used for the mid-point of the calibration graph) then the larger the aliquot required to give this amount the greater will be the weight of the ion concerned which the final solution will contain. The ions tested (and their levels as ppm. found by Vergnano in toxic material from Whitecairns) were:- aluminium, 100; iron, 100; manganese, 200; chromium, 1; zinc, 30; copper, 10; cobalt, 5; magnesium, 30; calcium, 70.

The table below shows tha effect produced by the addition of each ion on the Spekker reading equivalent to 0.05 mg. nickel in each aliquot.

TABLE 6

Effect of single ions on Spekker Readings

Ion	Aliquot (ml)	Reading	Ion	Aliquot	Reading	Ion	Aliquot	Reading
No ions added	--	58.1	--	--	--	--	--	--
Al	20 ml.	55.2	Cr	20 ml.	54.1	Co	20 ml.	54.0
	10	54.8		10	54.7		10	55.6
	5	54.5		5	54.8		5	54.3
Fe	20	54.3	Zn	20	54.7	Mg	20	54.3
	10	55.2		10	55.4		10	55.2
	5	54.5		5	55.8		5	54.8
Mn	20	51.5	Cu	20	54.6	Ca	20	54.6
	10	53.8		10	55.7		10	55.0
	5	54.7		5	54.7		5	55.2

The/

The lowest spekker reading of 51.5 is equivalent to introducing an error of about 15% in the final answer for nickel as ppm. in the dry matter. The effects produced by other ions were much smaller and would result in errors of between 2 and 10%. Presumably these effects would be constant for any one set of samples so that the interpretation of results would still be possible although the levels of nickel found would be somewhat higher than the actual nickel content.

Effect of all ions and increase in amount of citric acid used on spekker readings obtained with 0.05 mg. nickel

Three dry-matter extracts containing 50, 100 and 200 ppm. nickel were simulated by diluting 5 ml., 10 ml.,and 20 ml. of 1000 ppm. nickel solution to 100 ml. Aliquots (20 ml., 10 ml., and 5 ml.) containing 1 mg. nickel were next drawn off and diluted with an equal volume of the "all ion" solution. Then 20 ml., 10 ml., and 5 ml. aliquots were drawn off these composite solutions so that the weight of nickel present in each aliquot was 0.05 mg. although the total amount of interfering ions increased with size of final aliquot. The normal 10 ml. citric acid used was here increased to 20 ml. to find if this gave increased accuracy. Previous results had shown that 0.05 mg. nickel gave Spekker readings in the range 54.3 - 55.4, corresponding to a nickel content of 205 - 210 ppm. in the dry-matter for a 5 ml. aliquot.

As had happened with single ion additions, the maximum change in Spekker reading was produced when the 0.05 mg. nickel was present in 20 ml. of the composite solution. The effect

of/

of increase in citric acid with 5 ml. aliquots was to reduce the value found so that it fell within the range of values derived from Spekker readings produced from 0.05 mg. nickel. In the case of 10 and 20 ml. aliquots, the beneficial effect of additional citrate was less noticeable especially in the case of the larger aliquot.

TABLE 7

Interference effects due to all ions

Effective aliquot	Spekker reading vol. citric acid 10 ml.	20 ml.	Mean Spekker reading 10 ml.	20ml.	Nickel as ppm. in dry matter		% errors	
5	53.7	54.8	54.0	55.1	212	206	6	3
	54.2	55.4	--	--				
10	53.1	54.1	53.5	54.0	108	106	4	3
	53.8	53.8	--	--				
20	51.0	52.3	51.3	52.2	56	55	12	10
	51.6	52.0	--	--				

Conclusions

Satisfactory results have been obtained with this colorimetric method when used to determine the level of nickel in the dry-matter of nickel-toxic plant material and in extracts of soils of high nickel content. In the case of soil extracts, organic matter must first be destroyed. If applied to the determination of nickel in normal plant tissue or soil extracts low in nickel, the results would be less reliable due to the relatively greater levels of other interfering ions present in the extract.

SERPENTINE SOILS

REVIEW OF THE LITERATURE.

Serpentine soils may be described as soils of high magnesium content derived from serpentine rocks. They are of widespread occurrence throughout the world although the areas themselves may be of a quite localised nature. These widely scattered serpentine areas have several factors in common. In almost all cases they (1) are sterile and unproductive either for agricultural or silvicultural purposes (2) possess unusual floras characterised by narrowly endemic species and (3) support vegetation in striking physiognomic contrast to that on adjacent soil types.

Reference to the literature over the past fifty years, shows that serpentine vegetation areas have been described from almost every country in Europe, and reports from localities as far apart as Japan, Cuba, Puerto Rico, Canada and the United States show the widespread interest manifested in such areas. In the United States, quite large serpentine areas occur on both the Atlantic and Pacific seaboards. Scattered outcrops occur in Pennsylvania and Maryland while the Pacific coast states boast of much more extensive areas of serpentine. The Californian areas in the Sierra Nevada and the central and north coast ranges total several thousand square miles (Whittaker (144)).

The/

The reasons for the infertility associated with soils derived from serpentine (a magnesium silicate) were not immediately obvious. The fact that many serpentine soils are deep and of good colour and texture made the problem all the more puzzling. On the other hand, certain serpentine soils are shallow and consist of little more than relatively unweathered rock particles.

Since serpentine is a magnesium silicate it is not surprising that soils derived from it are high in that element. As a result of this the pH of these soils is generally alkaline even although they often occur in areas of relatively high rainfall, where losses of bases would be expected. The first of these factors appears to offer a basis for explanation, since at the beginning of this century, Loew (73) had introduced his concept of the calcium-magnesium ratio in plant nutrition. He advocated that one of the main functions of calcium in plant metabolism was to neutralise the toxic action of magnesium and that a certain calcium-magnesium ratio is necessary for the proper growth and development of the plant. Several writers, including Loew himself, suggested that the serpentine infertility was caused by abnormally high magnesium content. However, Lipman (71), in 1916, after a thorough resume of the extensive literature on the calcium-magnesium ratio hypothesis, concluded that the experimental results did not warrant the assumption of the existence of a critical ratio for a plant or group of plants. In fact, there seemed no more reason to believe in such a ratio

in/

in the soil that to suppose that critical ratios also existed between calcium and potassium or between calcium and any other essential element.

Even after Lipman's exposé of the concept, much work continued to be done to study the effect of different calcium-magnesium ratios in the soil on uptake of nutrients by various crop plants. The sort of results obtained can best be illustrated by reference to a recent paper by Sanik, Perkins and Schrenk (116) who investigated the uptake of phosphorus, potassium and certain essential trace elements by wheat and sorghum from soils in which the calcium-magnesium ratio was varied from 7:1 to 1:1. In the case of sorghum, variation in the calcium-magnesium ratio did not produce appreciable differences in the uptake of phosphorus and potassium. Wheat, however, absorbed less phosphorus and more potassium from soils of narrow calcium-magnesium ratio. For boron and manganese, sorghum again showed little change in the amounts absorbed at different calcium-magnesium ratios and the same was true for the absorption of boron by wheat, although for both plants there was a large increase in the amount of boron absorbed at the 1:1 ratio. Wheat was found to absorb least manganese from soils in which the ratio was 5:1 and most at ratios of 3:1 and below. While the narrowest ratio used in these studies is not uncommon in serpentine soils, ratios of 1:10 and higher are much more usual, suggesting that uptake of nutrients in such soils might well be modified by the proponderance of magnesium over calcium, although this in itself is unlikely to cause complete infertility.

Workers/

Workers anxious to explain the infertility of serpentine soils had perforce to turn to other possible causes. Gordon and Lipman (32) drew attention to the low levels of nitrate and phosphate ions in such soils and demonstrated that such low levels limited the growth of barley. They also suggested that the alkalinity of the soils contributed toward the infertility. Although the Californian soils used in their studies were alkaline, not all serpentine soils are alkaline. As Walker (137) points out, alkaline soils of other origins are often quite fertile and, in any case, alkalinity per se cannot serve as a general explanation for serpentine infertility. Lipman (72), at that time (1926), emphasised the unsatisfactory nature of the soil microflora, and later, Robinson, Edgington and Byers (112) pointed out that certain serpentine rocks are too low in alumina to form sufficient clay and this may explain the formation of only a thin soil mantle on many level serpentine areas in Maryland. After examining results of physical analyses carried out on various serpentine soils, the same authors concluded that, in general, serpentine soils possess no physical characteristic which would render them unsuitable for plant growth.

Analyses of serpentine soils and rocks had revealed that their heavy metal content was often high. In particular nickel, cobalt and chromium appeared to be present in very high concentration compared with the levels found in acidic soils and rocks. Geochemists had worked out the principles involved in the uptake of trace constituents from magmas by the/

the constituent minerals of an igneous rock in the process of crystallizing. In the incorporation of these trace constituents into a mineral, ions of these take the place of other ions of similar ionic radii. Thus it is found that Ni^{++}, Co^{++} and Cr^{++} occur in the place of Mg^{++} in minerals of basic igneous character because of their similar ionic radii. It therefore follows that serpentine soils normally high in magnesium would be expected to contain relatively higher concentrations of such heavy metals than their counterparts derived from acidic rocks of much lower magnesium content. Robinson et al.(112) were the first to propose that the infertility associated with certain types of serpentine soils could be due to their content of heavy metals such as nickel and chromium. They studied the Conowingo silt loams of Maryland and concluded that the levels of chromium and nickel were high enough to be the primary cause of the infertility. Birrell and Wright (14) reached the same conclusion regarding a serpentine soil from New Caledonia. In Italy, Pichi-Sermoli (104) and Minguzzi and Vergnano (92) ascribed toxicity symptoms in mountain flora to their high nickel content.

More recently workers in Japan (28) and Hawaii (18) have commented on the high level of nickel in their local soils, and Hunter and Vergnano (56) have reported the same for some Aberdeenshire soils. The table below sets out results for chromium and nickel analyses reported in certain of the papers mentioned above so that an idea of the amounts likely to cause toxicity in plants may be obtained. For comparison, values for a granitic soil from Aberdeenshire are also included.

TABLE 8

Nickel and Chromium Contents of Serpentine Soils

	Ni concentration (ppm.)			Cr concentration (ppm.)		
	Total	Acetic-soluble	NH_4Ac soluble	Total	Acetic-soluble	NH_4Ac soluble
Scotland						
Granitic soil (Mitchell (95))	10	-	-	5	-	-
Serpentine soil (Mitchell (95))	200	-	-	700	-	-
Serpentine soil (Swaine (125))	550	104	7.2	3500	0.33	0.10
Serpentine soils (Vergnano (132))	-	134-403	26-45	-	0.39-0.76	-
Serpentine soil*	1000	-	-	-	-	-
U. S. A.						
Conowingo Silt Loam (Robinson et al. (112))	157	-	1.8	1232	-	0.17
Brown serpentine loam (Hibbard (47))	-	64	-	-	-	-
New Caledonia						
Serpentine soil (Birrell and Wright (14))	1400	-	27	3.34%	-	9
Japan						
Serpentine soil, topsoil (Harada (33)) subsoil	2400	-	-	-	-	-
	2800	-	-	-	-	-
Puerto Rico						
Serpentine soil (51)	-	-	-	8550	-	-
Hawaii						
Serpentine soils (Chang and Sherman (18))	96-661	-	0.31-2.56	-	-	-

* Whitecairns basin soil. Analysis by Dr. D..J. Swaine.

A critical review of the literature on serpentine soils indicates that while heavy-metal toxicity may be the primary factor causing infertility in certain areas, it is unlikely to be solely responsible has a general rule. Some other factor or factors are involved and it was not until comparatively recently, using modern techniques and ideas, that the real reason for the widespread infertility of these soils emerged.

Vlamis and Jenny (135), on comparing the heavy-metal content of fertile and unproductive soils from serpentine areas in California found no consistent differences to account for the infertility. They concluded that the primary cause was the low calcium status of these soils, a suggestion later adequately confirmed by field experiments carried out by Martin, Vlamis and Stice (81). Massive applications of gypsum were used to increase the calcium status of the soil and yields of hay were found to be directly related to the degree of calcium saturation of the soil. After a number of years only the top 6 inches of soil had a sufficiently improved calcium status to bring about normal growth of agricultural crops. Another class of serpentine soil was noted by Walker (136) in California who found that poor growth still resulted even when the soil calcium status had been improved. Investigation showed that this soil was deficient in molybdenum.

At this stage it is convenient to compare the exchangeable cations in serpentine soils from various parts of the world with the levels found in some uncultivated Aberdeenshire soils, collected in the vicinity of serpentine outcrops.

TABLE 9

Exchangeable Cations in Serpentine Soils

Source of sample	pH	Exchangeable cations milliequivalents/100 g. soil Ca	Mg	K	Na	Ratio Ca : Mg
Scotland (Aberdeenshire)						
Auchenleith, surface soil*	6.6	3.3	23.0	0.64	0.70	1 : 7.0
Tombreac, 0-6 in. layer	6.5	5.1	25.7	0.62	0.48	1 : 5.0
6-12 in. layer	6.9	1.5	9.6	0.14	0.18	1 : 6.4
Greenhill, 0-6 in. layer	6.2	3.0	24.6	0.94	0.32	1 : 8.2
6-12 in. layer	7.2	0.9	9.6	0.13	0.12	1 : 10.7
Whitehillocks, 0-6 in. layer	6.8	1.2	9.3	0.14	0.20	1 : 7.8
6-12 in. layer	7.1	0.3	6.0	0.07	0.09	1 : 20.0
Breagach, 0-6 in. layer	6.7	1.4	28.5	0.70	0.19	1 : 20.4
6-12 in. layer	7.1	0.3	7.4	0.22	0.12	1 : 24.7
England (Cornwall)						
Lizard (Surface soil)	5.2	0.9	1.0	--	--	1 : 1.1
(Subsoil)	6.0	0.9	3.2	--	--	1 : 3.6
Norway						
Gudbrandsdalen, (Surface soil)	7.1	16.5	1.4	0.13	0.12	1 : 0.08
Sweden						
Lake Kultsjön, (Surface soil)	6.7	3.6	3.3	0.15	0.04	1 : 0.9
Italy						
Nr. Florence, (Surface soil)	4.9	0.3	0.1	0.05	0.02	1 : 0.4
U. S. A.						
Dublin, Maryland (112)	5.9	0.16	4.09	0.06	0.13	1 : 25.6
Chelan Co., California (137)	6.6	2.03	5.95	0.20	--	1 : 2.9
Lake Co., California (137)	6.8	2.12	12.1	0.11	0.05	1 : 5.7
Kale Co., California (137)	6.6	2.33	19.7	0.13	0.04	1 : 8.5

Figures in parentheses are literature references.

* Analyses not otherwise credited were supplied by Dr. H.G.M.Hardie, Analytical Section, Macaulay Institute for Soil Research, to whom I am indebted.

In normal agricultural soils in Aberdeenshire derived from acidic rocks, the calcium-magnesium ratio is normally about 1:0.1 Amongst the serpentine soils listed above, only the Norwegian soil has a ratio of this order. The Lizard serpentines are higher in calcium than would be expected presumably due to limestone intrusions. The pH values for the Aberdeenshire soils are all above 6.0, the subsoil values being higher than those for the topsoil, which was generally peaty in character in these uncultivated soils. For topsoils, ratios of 1:5 to 1:10 are found with the exception of that from Breagach vicinity which has a value of 1:20.4. More magnesium is found in the subsoil samples and this gives a range of values from 1:10 to 1:25, that for the Tombreac sample being lower (1:6.4).

Acetic-soluble and "exchangeable" nickel values for these samples were generally lower than values obtained by Vergnano from the Whitecairns area, as the table below shows.

While soil-culture experiments, using the oat plant as an indicator, offer the best method of testing for the presence of heavy metal toxicities in soils, certain conclusions can be drawn from the analytical data given in Table 10. From our knowledge of the behaviour of the Whitecairns soils, only the Breagach topsoil would be expected to cause nickel toxicity in oat plants grown on it. The levels of "exchangeable" nickel, which appear to govern the degree of toxic symptoms, appear to be too low in the other samples.

T A B L E/

TABLE 10

Nickel contents of Aberdeenshire Serpentine Soils

Source of Sample	pH	ppm. nickel in soil Acetic soluble	"Exchangeable"
Whitecairns	5-7	134 - 403	26 - 45
Auchenleith	6.6	126	12.6
Whitehillocks	6.8	90	11.2
	7.1	40	10.6
Tombreac	6.5	114	10.8
	6.9	36	9.4
Breagach	6.7	285	41.3
	7.1	188	14.3
Greenhill	6.2	88	14.8
	7.2	46	8.9

EXAMINATION OF THE SOILS AT WHITECAIRNS

The Whitecairns area, where the abnormal oat plants were found, consists of a poorly-drained basin about 4 miles long and 2 miles wide surrounded by low hills. The principal rock type is a dark, greenish-black, massive serpentine with talc and chlorite veining, outcrops of which occur on the upper hill slopes. This serpentine rock predominates in the mixed drift from which the soils are derived. The soils belong to the Leslie association (Glentworth (30)) and are of two main types - a loam or peaty loam on the hill slopes ("hill" soil) and a peaty soil in the basin ("basin" soil). Oats growing on the basin soils were more severely affected than those growing on the hill soils.

Vergnano (132) first examined both soil types in order to reveal any factor likely to be responsible for the toxic symptoms seen in the oats. Nickel was found to be abnormally high in both soils while cobalt and chromium were also higher than usual. As was to be expected, the magnesium content was high, manganese, calcium and potassium, normal and phosphorus, low. Molybdenum did not appear to be present at a level likely to cause deficiency. Results of plant analyses confirmed these soil findings and the conclusion drawn was that nickel (and possibly cobalt) is present in these soils at toxic levels, and therefore that the infertility is primarily due to nickel toxicity. There was good correlation between the nickel content of the plant and its necrotic symptoms. There was

a/

a correlation between nickel level in the soil (either acetic-soluble or exchangeable) and degree of necrotic symptoms, but only when soil pH differences were small. Nickel uptake and soil pH appear to be indirectly related, uptake being greatest from the more acid soils of the basin type.

Next, the symptoms produced in sand-cultured oats, receiving various levels of nickel in the nutrient solution, were examined and were found to be identical with those seen in plants from the Whitecairns area. It was found that, for the particular nutrient solution used, a concentration of 2.5 ppm. nickel in the solution would produce symptoms of comparable severity to those seen in the field. The same effects could be produced by adding nickel solutions to soils of negligible nickel content although the uptake of nickel from such soils depended to a great extent on their pH and general nutrient status. In particular the level of calcium present in the soil exercised a marked beneficial effect. This confirmed earlier findings on similar soils, when liming had been shown to reduce the uptake of nickel markedly. (Mitchell (93)).

Further experiments by Vergnano showed that gypsum did not exert the same beneficial effect as lime and therefore, the effect appeared to be due to a change in soil pH rather than to an increase in the exchangeable calcium which could then by competition, reduce the absorption of nickel. Further experiments carried out to study this effect in more detail are described below.

Effect of change in soil pH on uptake of Nickel

The basin soil was chosen for these experiments in preference to the hill soil, because of its more satisfactory manganese status. Two similar experiments were carried out; in the first calcium carbonate was used to bring about change in soil pH, in the second sodium carbonate was used. The initial soil pH was 4.8 - 5.0 and 'lime requirement' curves were produced (using Ca^{++} or Na^{+}) to ascertain the amounts of calcium or sodium needed to raise the pH in 0.5 pH unit steps to an upper limit of 7. A basic NPK dressing* was incorporated in the soil at the same time as the liming material. A similar series of limed pots received twice the basal dressing in order to study the effect of increased rate of supply of nutrients on the uptake of nickel. Two 9 in. pots per treatment were used and there were thus ten treatments in all. After the soils had been limed and fertilizers added, the pots were filled and watered for 7 days before sowing the seed. The soil pH of each treatment was determined before and after the experiment, together with the level of exchangeable nickel in the soils (see Table 11). After germination, plants were reduced to 80 per pot and growth was allowed to proceed for 35 days before harvesting.

* N (as $(NH_4)_2SO_4$) 5 g.; P (as superphosphate) 6 g.; K (as KCl) 2.5 g.; all per pot.

In both series toxic symptoms of moderate severity* appeared in the control plants after about 10 days but no symptoms appeared in any of the limed pots although as analysis showed, the uptake of nickel by these plants was still quite high. On harvesting, yields of the two pots in each treatment were combined, and fresh-tissue extracts were made of mid-stems, while fully expanded leaves were dried and their nickel and iron contents determined. The results are set out in the tables below.

* In assessing toxicity symptoms, the same system of scoring was always followed. The ratings used were as follows: healthy (0), low symptoms (L), moderate or medium symptoms (M), high or very high symptoms (H) and (VH). Further differentiation is indicated by the use of plus or minus signs.

TABLE 11

Soil and plant analyses for $CaCO_3$ series.

(Treatment 662 - 666 low fertilizer dressing)
(Treatment 667 - 671 high fertilizer dressing)

Treat. No.	Soil pH		Exch. Nickel	Fresh yield per 100 plants (g.)	Concentration (ppm.) in fresh tissue extract					Concentration (ppm.) in leaf dry matter		Necrotic Symptoms
	Initial	Final			NO_3	PO_4	K	Ca	Mg	Fe	Ni	
662	4.9	5.2	55.0	64.8	124	6.2	328	57.2	7.9	58	188	M
663	5.9	5.7	51.5	74.4	117	2.8	328	37.2	8.4	56	102	0
664	6.4	6.4	38.8	110.7	130	2.1	328	37.2	7.1	63	98	0
665	7.0	6.8	31.0	92.3	165	1.5	309	39.2	7.4	80	75	0
666	7.3	7.1	25.6	92.4	176	1.9	274	39.2	7.5	104	77	0
667	4.9	5.1	54.0	87.2	128	11.7	368	29.2	6.2	65	185	M
668	5.6	5.5	45.6	109.6	180	6.0	378	34.0	7.2	137	128	0
669	6.1	6.0	41.8	102.4	256	5.9	400	43.6	7.4	92	98	0
670	6.8	6.7	28.0	109.4	277	3.6	343	39.2	6.7	76	80	0
671	7.2	7.0	25.3	128.5	273	3.3	338	42.0	7.3	84	75	0

TABLE 12

Soil and plant analyses for Na_2CO_3 series

(Treatments 702 - 706 low fertilizer dresing)
(Treatments 707 - 711 high fertilizer dressing)

Treat. No.	Soil pH		Exch. Nickel	Fresh yield per 100 plants (g.)	Concentration (ppm.) in fresh tissue extract					Concentration (ppm.) in leaf dry matter		Necrotic Symptoms
	Initial	Final			NO_3	PO_4	K	Ca	Mg	Fe	Ni	
702	4.8	4.2	40.3	73.0	120	4.5	362	37.6	10.0	117	114	M
703	5.7	4.6	29.8	99.2	212	3.7	315	24.8	9.8	122	109	0
704	6.2	5.1	31.8	93.9	216	4.1	291	16.4	9.5	107	79	0
705	7.0	6.0	24.3	74.5	152	3.3	210	7.6	7.2	103	49	0
706	7.5	6.8	24.0	83.4	128	3.0	131	5.2	4.3	92	40	0
707	4.7	4.3	40.6	100.2	70	8.4	338	24.8	7.6	125	113	M
708	5.4	4.5	38.8	87.5	168	9.1	304	20.0	7.0	115	96	0
709	5.9	4.5	37.6	95.0	190	8.7	248	18.8	8.4	115	84	0
710	6.6	5.3	34.6	76.7	224	9.4	184	14.0	6.6	115	58	0
711	7.2	5.9	20.8	76.7	229	7.0	143	8.4	5.4	96	48	0

The limited nature of these experiments preclude any definite conclusions regarding yield or major-element composition of the plants; however it is worth noting the similar effect that both lime and sodium carbonate have had in reducing yield at pH levels above 6.0 unless an adequate supply of other nutrients was present. The results, however, clearly bring out the effect which increase in soil pH, brought about either by Ca^{++} or Na^{+}, has on the exchangeable nickel in the soil and the uptake of nickel by oats from these limed soils. Exchangeable nickel and uptake of nickel fall with increasing soil pH. The mechanism underlying this effect is not evident but it seems likely that a change in the form of the soil nickel is produced by the pH variation. Krotov (66) has noted that nickel was precipitated in soil at a pH between 6 and 7 while Oertel, Prescott and Stephens (101) found that nickel uptake by clover was greatest from soils of acid pH.

While these pot experiments were being conducted, the recovery of added nickel from soils of various pHs was being studied. The soils used were from the Countesswells Association which is derived from granite and probably contained less than 2 ppm. acetic-soluble nickel(Mitchell (95)). Three experiments were carried out, differing in details although the experimental method used was the same in each case. To 20 g. lots of air-dried soil was added 4 mg. nickel in solution together with enough water to bring the soil to 30% moisture capacity. The soils were stored moist for 10 days and then/

then allowed to dry out completely, lumps being broken down as they formed by means of a glass rod. The soils were then leached in the usual way with neutral N-ammonium acetate to determine exchangeable bases and the recovery of the added nickel. Each treatment was set up in duplicate; agreement between duplicates was good, and the mean values are given in the tables below.

Experiment 1

Four bulk samples of soil were taken from a long-term liming experiment. The soils were drawn from plots receiving (A) no lime, (B) 17.5 cwt. (C) 35 cwt. (D) 52.5 cwt. ground limestone per acre.

TABLE 13

Effect of degree of calcium saturation on recovery of added nickel

		Exchangeable bases (me./100 g. soil)					Nickel (mg.)	
Soil	pH	H	Ca	Mg	K	Na	Recovered	Fixed by Soil
A	5.15	10.3	2.85	0.06	0.13	0.13	2.34	1.66
B	5.50	7.9	4.20	0.06	0.11	0.14	2.20	1.80
C	5.75	7.4	5.40	0.07	0.15	0.16	2.14	1.86
D	6.20	5.9	8.40	0.10	0.11	0.22	1.65	2.35

Experiments 2 and 3

Using soil A (pH 5.2) a 'lime-requirement' curve was constructed to find out amount of (a) $CaCO_3$ (Expt. 2) or (b) Na_2CO_3 (Expt. 3) which would be required to raise the soil pH to

to pH 7 in 0.5 pH unit steps. In practice it was found more convenient to "round off" these calculated amounts and measure the actual pH arrived at in a series of controls carried through the same procedure but without added nickel. The results of these experiments are given below.

TABLE 14

Recovery of nickel from limed Craigiebuckler soil

$CaCO_3$ added (mg.) per 20g. soil	Final pH	Exchangeable cations (me./100g. soil) Ca	Mg	K	Na	Recovery of nickel (mg.) Series C	Series D
0	5.10	3.00	0.06	0.14	0.12	2.07	2.09
30	5.65	5.40	0.05	0.15	0.12	1.85	1.98
60	6.15	7.65	0.05	0.17	0.14	1.59	1.58
100	6.60	10.35	0.04	0.18	0.12	1.18	1.14
140	6.85	13.65	0.06	0.19	0.12	0.85	0.84
210	6.80	19.65	0.06	0.22	0.14	0.63	0.63

TABLE 15

Recovery of nickel from limed (Na_2CO_3) Craigiebuckler soil

Na_2CO_3 added (mg.) per 20g. soil	Final pH	Exchangeable cations (me./100g. soil) Ca	Mg	K	Na	Recovery of nickel (mg.) Series C	Series D
0	5.2	3.00	0.13	0.19	0.12	2.15	2.22
10	5.7	3.20	0.06	0.22	0.94	2.11	2.11
20	6.1	3.00	0.06	0.23	1.78	2.13	2.08
40	6.6	3.20	0.08	0.26	2.88	1.95	1.93

These/

These laboratory experiments gave results similar to those obtained in the pot-culture work. Increase in soil pH, whether brought about by calcium or sodium carbonate was found to reduce the amount of nickel extracted or recovered from the soil by N-ammonium acetate.

THE ROLE OF NICKEL IN PLANT GROWTH

Although it is not at present accepted that nickel is essential for healthy plant growth, various authors have undertaken studies designed to support or refute this contention. Thus, Arnon (2) suggests that under special circumstances nickel may be necessary or helpful to plants, whilst Roach and Barclay (111) consider it an essential element. More recently, Fujiwara and Kikuchi (28) have contended that nickel is necessary for the healthy growth of soybean, but Hewitt and Bolle-Jones (46) using a range of crops grown in highly purified media, were unable to support this claim, but, as they pointed out, if the element is essential, then the deficiency threshold probably occurs at very low concentrations.

On the other hand, it has long been recognized that nickel is highly toxic to plants and the following table summarises the results obtained by various workers using sand- or solution-culture techniques.

Instances of plant injury following the application of solutions of nickel salts to the soil are cited by Petri (103) for olives, Loew (74) for cereals, Nicholas (97) for tomatoes and Vanselow (131) for citrus. In this connection, damage is often caused to agricultural crops on low-lying ground near industrial areas when rivers carrying industrial effluents containing quantities of heavy metals such as nickel and chromium overflow their banks.

The/

Authors	Plants used	Toxic level (as ppm. in Solution)
Brenchley (16)	Barley & beans	2 - 15
Cotton (21)	Buckwheat	2 - 60
Coupin (22)	Wheat, etc.	low
Haselhoff (34)	Beans & maize	2 - 40
Hewitt (41, 42)	Oats, sugar beet etc.	15 - 30
Jensen (62)	Wheat	7 - 15
Mazé & Mazé (82)	Maize	low
Millikan (89,90)	Flax, etc.	0.5 - 5
Scharrer & Schropp (117)	Oats, etc.	3 - 300

The typical symptoms produced by excess nickel in oats have been described by Scharrer and Schropp (117) and Hewitt (42) as white necrotic areas on the leaves, often appearing as longitudinal stripes. The latter author also points out that these specific symptoms are accompanied by an induced iron deficiency. Vergnano (132) describes the sequence of events in oat plants supplied with nickel as follows:

With low concentrations of nickel (1 or 1.5 ppm.) a slight mottling appears at the tip of the first leaf after about eight days. Within twelve hours this mottling is replaced by longitudinal alternate green and white striping of the leaf, which extends downwards from the tip, while other necrotic areas often appear in the mid-third of the leaf. While necrosis is becoming more severe, the second leaf is developing; it/

it usually remains normal but may become chlorotic. The young leaves which develop later are usually normal in appearance. Higher concentrations of nickel (2, 2.5 or 5 ppm.) produce necrosis within 4-5 days of germination; the same sequence of events is followed as was described above. Leaves which develop later are diffusely chlorotic. Longitudinal white areas appear simultaneously in the upper and mid-leaf regions and often spread over the whole width so that the leaf becomes devoid of pigment in such areas. The green areas which alternate with the white are generally healthy in appearance although sometimes chlorotic. These higher nickel concentrations produce stunting of the plants together with narrowing of the second and subsequent leaves.

Reference to the literature on the effects of heavy metals in excess on plants shows that this induced iron deficiency is a feature common to all such cases. Furthermore, each metal produces specific toxic symptoms in addition to this iron deficiency. Hunter and Vergnano (57) examined the effects of excess nickel, cobalt, chromium, manganese, zinc and copper on oats grown in sand culture and described the visual symptoms. It was noted that only cobalt produced toxic symptoms likely to be confused with those of nickel.

The elements were arranged in order according to their ability to produce chlorosis (iron deficiency) as follows:

$$Ni > Cu > Co > CrO_4 > Zn > Mn$$

This order differed from that found by Hewitt (42) using sugar beet

$$Co > Cu > Zn = CrO_4 > Ni > Mn$$

although it agreed quite well with the order of stability of organo-metallic complexes quoted by Hewitt (44) in a recent review article, in which he drew attention to the similarity between the order of stability of these complexes and that based on the ability of the metals to induce iron deficiency. This will be referred to later in connection with current theories on iron deficiency.

Vergnano next studied the effect of substrate pH on the absorption of nickel in a solution-culture experiment using ferric citrate at 1.2 ppm. as iron source. The cultures were maintained at pH values from 4.0 - 7.5 in 0.5 pH unit steps, and nickel (at 1.2 ppm.) was supplied to one series and withheld from another. At the end of 15 days when the experiment was harvested, all the 0-Ni cultures were healthy, irrespective of pH, while the +Ni cultures exhibited typical toxicity symptoms. The level of necrosis was the same over the whole pH range although the rate of development had varied, while cultures of pH 6 and above were chlorotic in addition to being necrotic. This suggested that the availability of iron had been lowered at these high pH values but the fact that the 0-Ni plants had remained healthy refuted this theory. It was therefore concluded that the chlorosis resulted from an upset in iron metabolism brought about by nickel.

Nickel absorption at various pH values with iron supplied as Fe-EDTA.

Chelate formsof iron have recently been finding wide use, particularly in U. S. A., in soils where iron nutrition of crops presents a problem (69, 124, 138, 139). The chelating agents are generally based on EDTA (ethylenediamine tetra-acetic acid) or its analogues, and Jacobson (61) first pointed out their usefulness as iron sources in solution-culture work. Iron always presents difficulties in such work because of its lowered availability with the passage of time or due to pH change of the medium. Ferric chloride can be expected to precipitate out in a few days while some improvement can be achieved by the use of ferric citrate or tartrate (108). Many workers prefer to add iron frequently in small amounts, while the iron supply in solutions of the type evolved by Crone depends on the small but constant amount supplied by a relatively insoluble iron salt, such as ferric phosphate.

A test of the chelate form of iron against citrate showed that, at pH below 6, uptake of iron by oat plants was practically the same from both sources. Above pH 6, however, there were indications that the chelate was a more efficient supplier of iron. As a result, it was decided to repeat Vergnano's pH experiment using Fe-EDTA instead of ferric citrate. At the time it was not known whether the Fe-EDTA chelate would dissociate in the presence of the ionic nickel in the nutrient solution/

solution and for the nickel complex to be formed. Reference to the literature suggested that such an effect might be feasible in pure solution but there was no evidence of any such replacement in the course of this or any subsequent experiment where this chelate form of iron was used. From later work which will be described in another section, it will be shown that, had the nickel chelate been formed to any extent in the nutrient solution, then the uptake of nickel would have been much reduced. The pH range 4 to 7 was covered in steps each of 1.0 pH unit and there were three crocks per treatment making a total of 24 crocks. Solutions were changed every 4 days and the experiment ran for 15 days. The same sequence in the onset of symptoms was noted as had been recorded by Vergnano. Necrosis appeared sooner at high pH values and developed more quickly. Chlorosis appeared after necrosis and then only in cultures of pH 6 and 7. Once again at harvest all 0-Ni cultures were healthy irrespective of pH and showed no signs of iron deficiency. Necrosis was of moderate severity in all crocks receiving nickel while portions of the leaves of plants at pH 4 and 5 which were not necrotic remained a healthy green. As mentioned above, chlorosis was severe at pH 6 and 7 in the plants supplied with nickel. Fresh-tissue extracts of stems were made on harvesting, and nickel and iron were determined in the leaf dry-matter. The table below sets out the results.

TABLE 16

Effect of pH on absorption of nickel in presence of Fe-EDTA.

Treat No.	pH of nutrient solution	Concn. (ppm.) in nutrient solution Ni	Fe	Fresh weight (g.) per 100 plants	ppm. in stem extract NO_3	P	K	Ca	Mg	(ppm.) in leaf dry matter Ni	Fe	Toxic symptoms Chlorotic	Necrotic
712	4.0 - 4.5	0	1.2	24.4	309	17.6	370	25.6	7.2	4	83	0	0
713	5.0 - 5.5	0	1.2	26.8	323	15.8	375	27.6	6.9	-	76	0	0
714	6.0 - 6.5	0	1.2	27.5	323	17.2	375	22.8	7.6	-	96	0	0
715	7.0 - 7.5	0	1.2	28.6	348	20.8	410	23.2	9.0	-	72	0	0
716	4.0 - 4.5	1.2	1.2	21.6	304	19.0	370	28.0	7.1	65	93	0	M
717	5.0 - 5.5	1.2	1.2	19.1	328	19.6	389	26.4	8.1	83	210*	0	M
718	6.0 - 6.5	1.2	1.2	18.6	323	27.0	400	26.4	9.4	110	91	H	M
719	7.0 - 7.5	1.2	1.2	22.9	297	24.0	360	26.4	9.9	100	273*	H	M

* unexplained abnormal values; ? contamination.

Comparison of the results obtained in these two experiments which differed only in the form of iron supplied, reveals little difference in the major-nutrient composition of the plants. While the same scores (for necrosis and chlorosis) were given in both experiments, interesting differences in the uptake of nickel and iron were found as the table below will show. The concentration of iron found in the control plants in each pH range is shown within brackets.

TABLE 17

Comparison of absorption of nickel and iron in different experiments

Iron source -	Ferric citrate				Fe-EDTA			
	(Elements as ppm. in leaf dry-matter)							
pH range	Fe		Ni	Ni/Fe	Fe		Ni	Ni/Fe
4.0 - 4.5	91	(132)	62	0.68	93	(83)	65	0.70
5.0 - 5.5	100	(111)	81	0.81	--	(76)	83	--
6.0 - 6.5	62	(94)	104	1.68	91	(96)	110	1.20
7.0 - 7.5	70	(98)	153	2.19	--	(72)	100	--

While the concentration of iron in control plants supplied with Fe-EDTA varies only slightly over pH range 4 - 7, the concentration in citrate-cultured plants at pH 4 is much higher (132 as against 83 ppm.) yet falls rapidly with increase in pH, until at pH 7 its value is only 98 ppm., although this is still higher than that of the corresponding Fe-EDTA culture. At each pH value, nickel causes a reduction in the concentration of iron in the plant. This is clearly shown by the results for the/

the ferric citrate series, those for the Fe-EDTA series being incomplete. However, the absorption of nickel at pH values 4 - 6 is similar in plants of both series, although a much higher value is found in the citrate series at pH 7. The nickel-iron ratio in the plant is of the same order for both series at pH 4, and the much lower value of the ratio found at pH 6 in the Fe-EDTA series confirms that this form of iron is more freely available to plants at pH values of 6 and above.

RATE OF ABSORPTION OF NICKEL BY OAT PLANTS

As the investigation proceeded it became evident that a knowledge of the rate at which nickel was absorbed by oat plants would prove most useful in interpreting results. It had been noted, both in the field and in sand-culture experiments, that, as might be expected, symptoms were most severe when plants were young but that they became less severe as the plants neared maturity, until latterly, plants which had been necrotic and very chlorotic assumed a relatively healthy appearance except for the presence of isolated necrotic areas on basal and intermediate laminae. This suggested that there was a change in the rate of absorption of nickel or that some other factor affecting toxicity was itself altered with time.

Relatively little appears to have been published with regard to the rate of absorption of nutrients by cereals but Russell (114) quotes work to show that the uptake of the nutrients nitrogen, phosphorus and potash follows the dry-matter production curve which is sigmoidal in character. No information on the concentration of nutrients in the plant at various stages of growth was available.

It was thought that the matter could best be studied by growing oats in sand-culture supplied with the basic nutrient solution and sampling these at intervals of 3 - 4 days from germination until the grain had emerged. A minimum of 4 g. of/

of dry-matter was required for analysis. The yields of dry-matter from oat plants from one 9 in. pot will reach this figure when plants are about 25 - 30 days old. It was seen that 8 - 10 pots per sampling would be required in the early stages of the experiment. The number required would fall quite rapidly to 4 - 5, then to 2 - 3, and finally one pot would suffice.

The experiment was carried out in the late spring in the greenhouse. As each age group was sampled, the number of plants in the sample was counted and the weight of dry matter obtained. Analysis was carried out for major elements, iron and nickel. The analytical data will be found in the appendix as Table 2. Yield figures are expressed as dry matter produced per 100 plants. The concentration figures are given rather than total uptake of each nutrient since a plot of uptake values against age of plant showed that in all cases a curve of sigmoidal type was obtained.

Table 2 shows how the toxic symptoms changed with the passage of time. Necrosis, once established, remained fairly constant but chlorosis (of young leaves) increased in severity until the plants were about 40 days old, then decreased until no chlorosis was evident in unfolding young leaves. These symptoms are reflected in the concentration of nickel in the plants which increases linearly for about 30 days, then falls off slowly for the rest of the period under investigation (73 days); its concentration at the end of that time being about two thirds of the maximum reached. Iron values on the other hand, show remarkably little variation with time. The maximum concentration

is/

is found in the youngest plants and the level in the plants decreases with age. This decrease is relatively slow and only involves a drop of about 30 ppm. although certain intermediate values drop to values which show a wider divergence from the maximum.

Although lack of greenhouse space precluded a complete duplication of this experiment by a second series, also sampled periodically, but receiving no nickel in the nutrient solution, it was possible to have a few pots of this type to enable comparisons of major nutrient content in the presence and absence of nickel to be made at various stages of growth.

TABLE 18

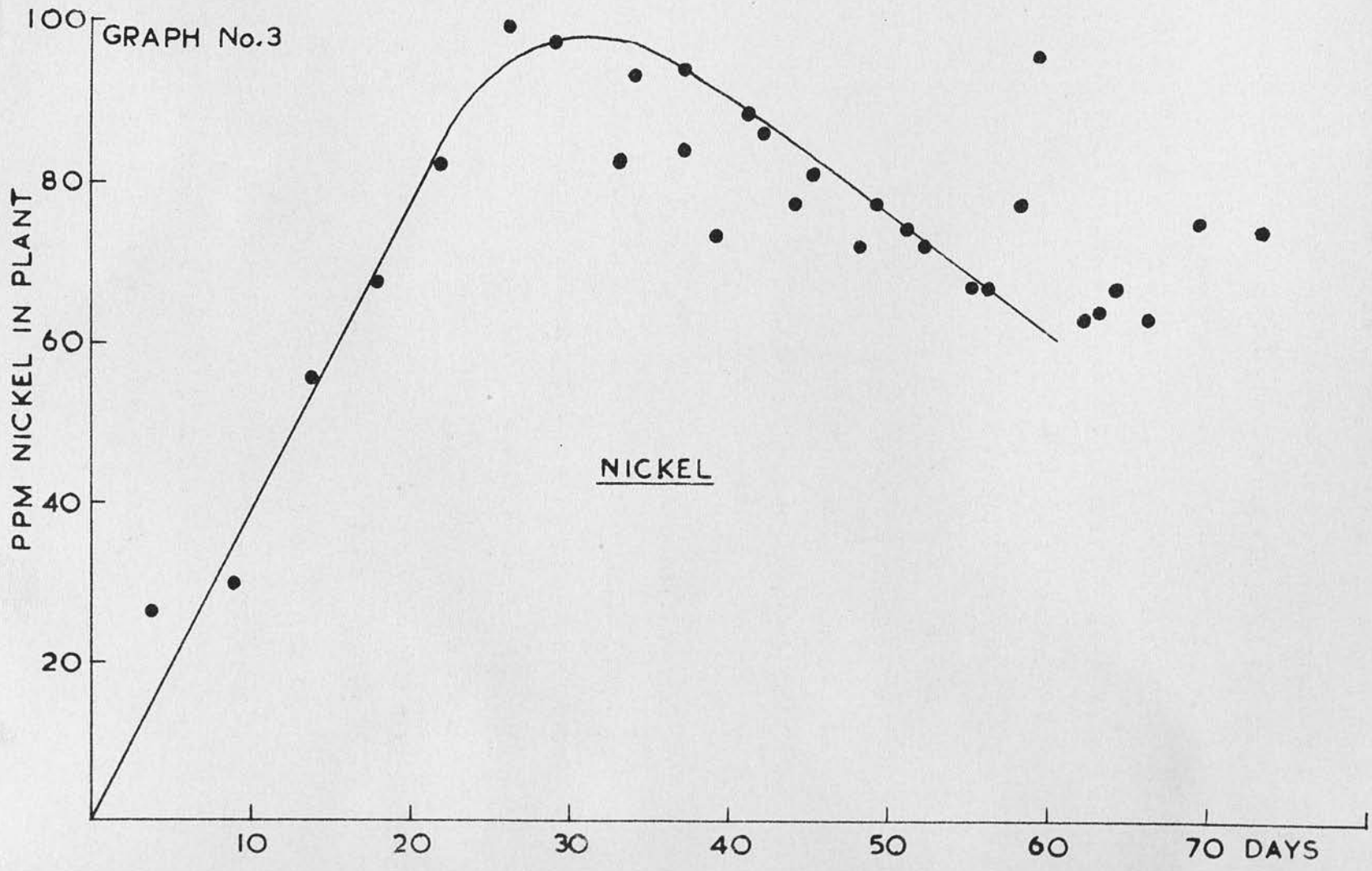
Levels of nutrients in healthy oat plants at various stages of growth

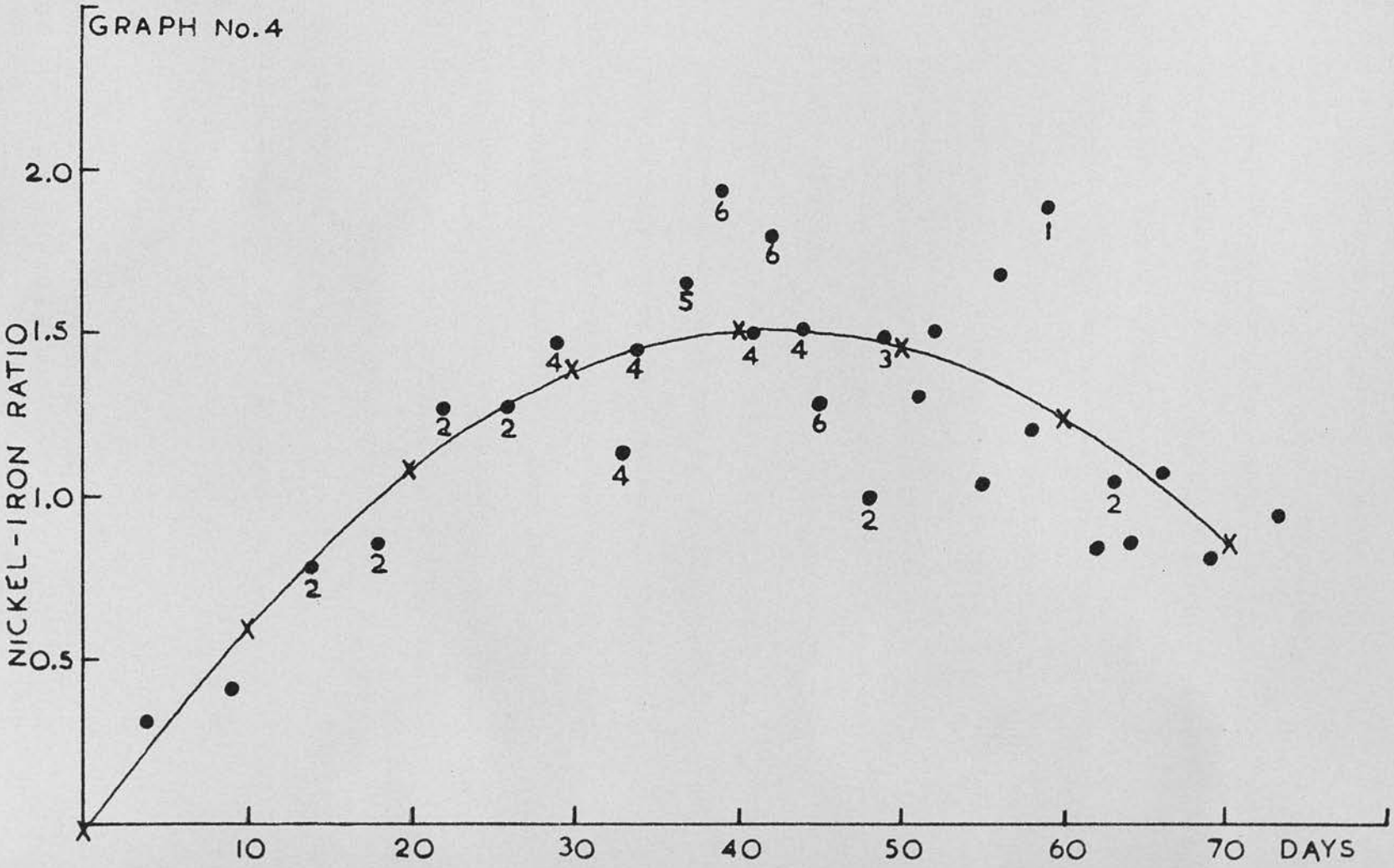
Treatment No.	Age of plant (days)	Percentage in dry matter				ppm. in dry matter
		P	K	Ca	Mg	Fe
853	6	0.748	1.71	0.495	0.318	86
854	10	0.660	2.78	0.583	0.338	80
855	14	0.570	3.36	0.518	0.338	82
856	28	0.520	4.70	0.308	0.265	69
857	36	0.495	4.79	0.343	0.285	67
858	44	0.348	3.88	0.275	0.204	66
859	47	0.355	4.10	0.308	0.263	75
860	54	0.320	4.18	0.308	0.250	70

The values obtained showed that, in general, nickel was having no great effect on the uptake of iron or major nutrients. The concentration of iron and phosphorus was always higher in control plants than in nickel-toxic ones of comparable age. The concentrations of magnesium and potassium were not dissimilar in plants at the same stage of growth. Potassium levels were identical in both sets for the first 30 days, thereafter the nickel-toxic plants had a much lower content, while that of the controls decreased at a much slower rate. The results for calcium were not too clear, but again the concentrations in control plants were generally lower than those of nickel-toxic plants, which agrees with the results of later experiments (see p.153).

Graphs have been prepared showing how the concentration of nickel varies with time in the nickel-toxic plants (Graph No. 3), and how the nickel-iron ratio in the plant changes with age of plant (Graph No. 4). The iron values obtained did not lend themselves to graphical treatment, although it was clear that the slope of the graph was very small. Calcium also gave erratic values in the later stages of growth which were not suitable for graphing. The concentration of calcium in the plants increased smoothly for 20 days to reach a maximum of 0.66%, then fell sharply to about 0.4%, the values varying irregularly about this level from then onwards.

The/


GRAPH No.3
100
80
60
40
20
PPM NICKEL IN PLANT
NICKEL
10
20
30
40
50
60
70 DAYS

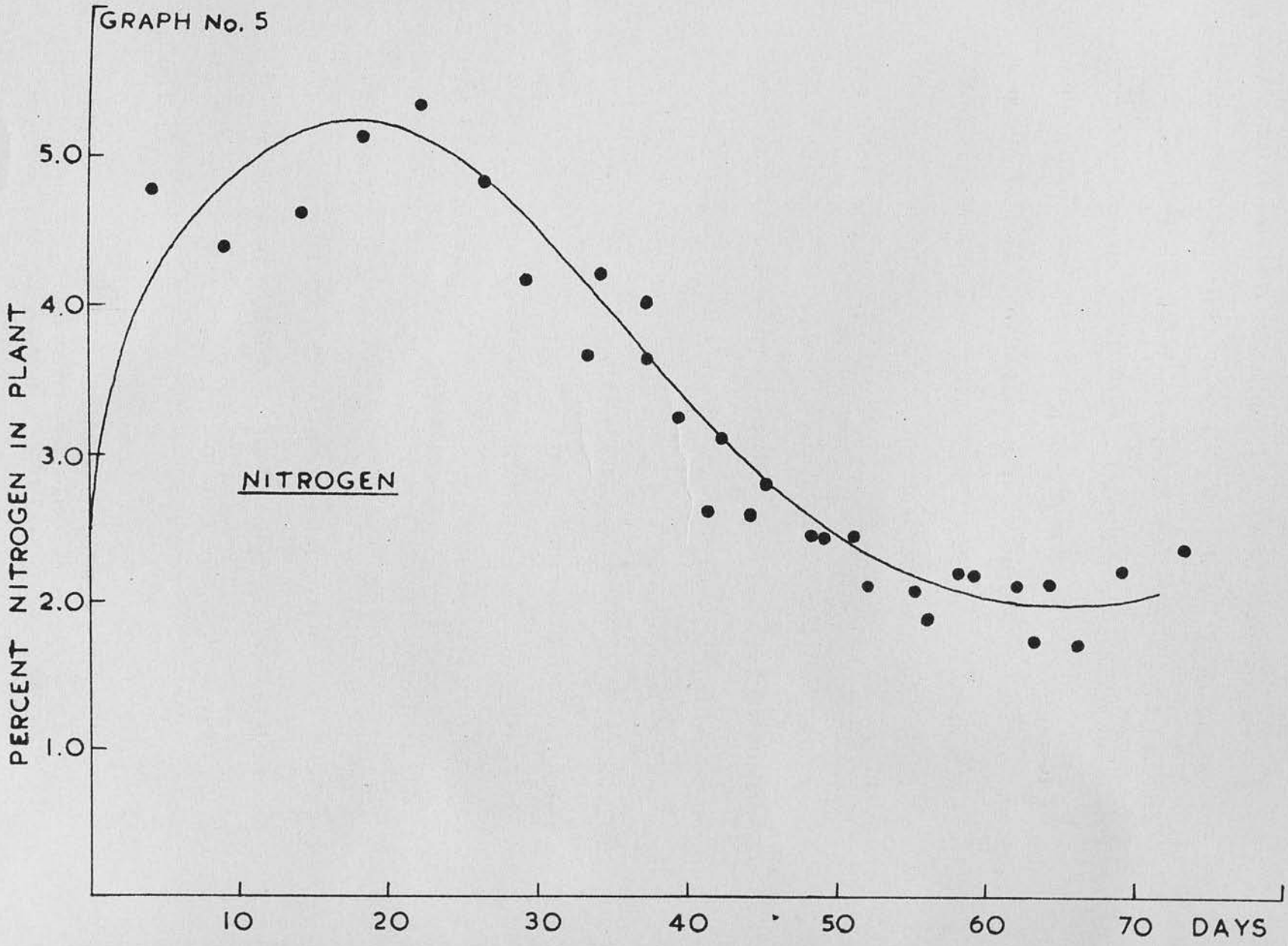

GRAPH No. 4
NICKEL-IRON RATIO
2.0
1.5
1.0
0.5
10
20
30
40
50
60
70
DAYS

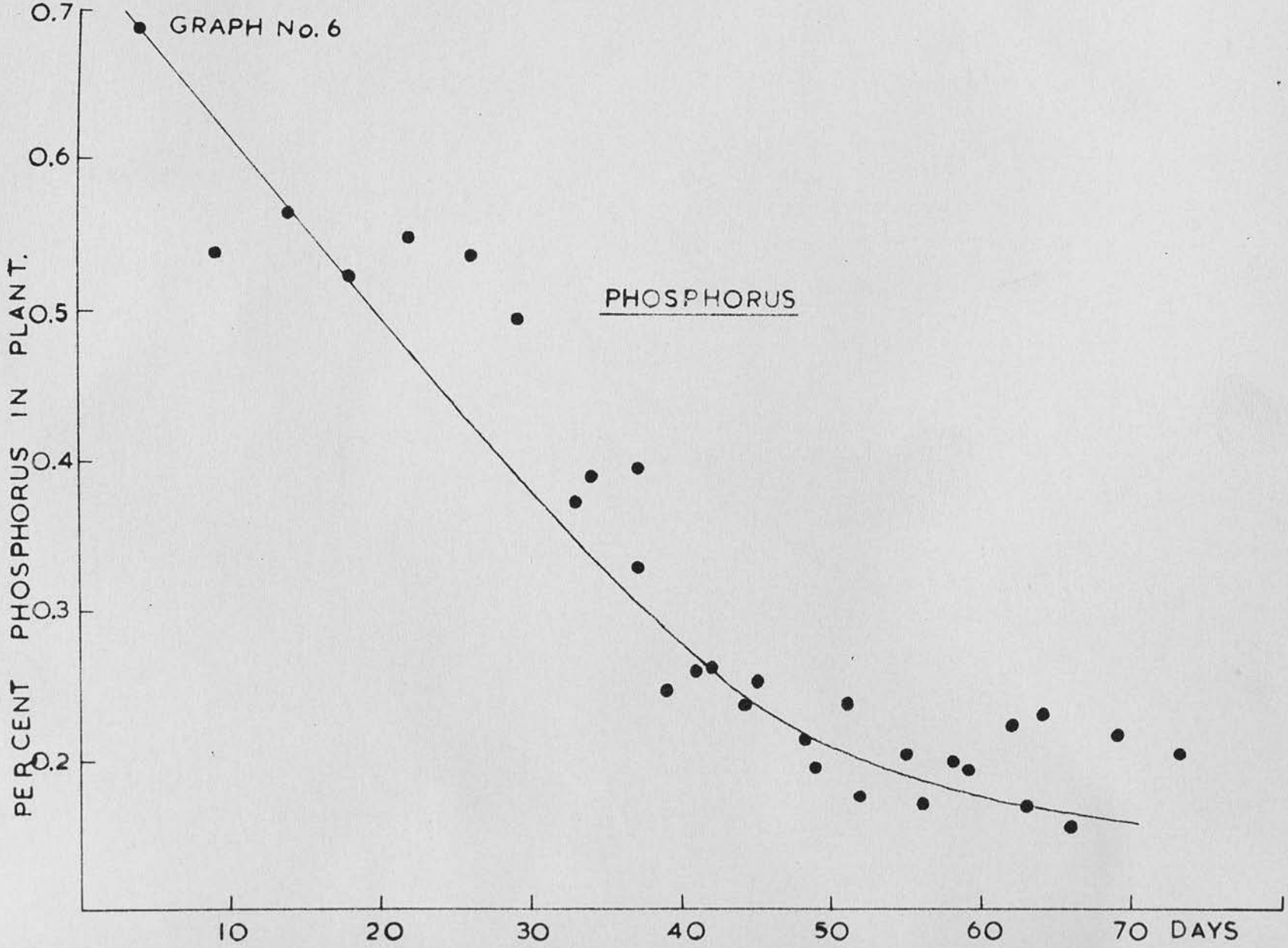
The curves for nitrogen, phosphorus, potassium and magnesium proved more amenable to treatment (Graph numbers 5 - 8). The phosphorus concentration starts at a high value and falls gradually with time whereas the other three elements increase to a maximum (at 18, 25 and 17 days respectively), and then decrease slowly. These changes are probably not a reflection of nickel treatment but rather of normal metabolic processes in the plant, associated with translocation of nutrients to the developing grain.

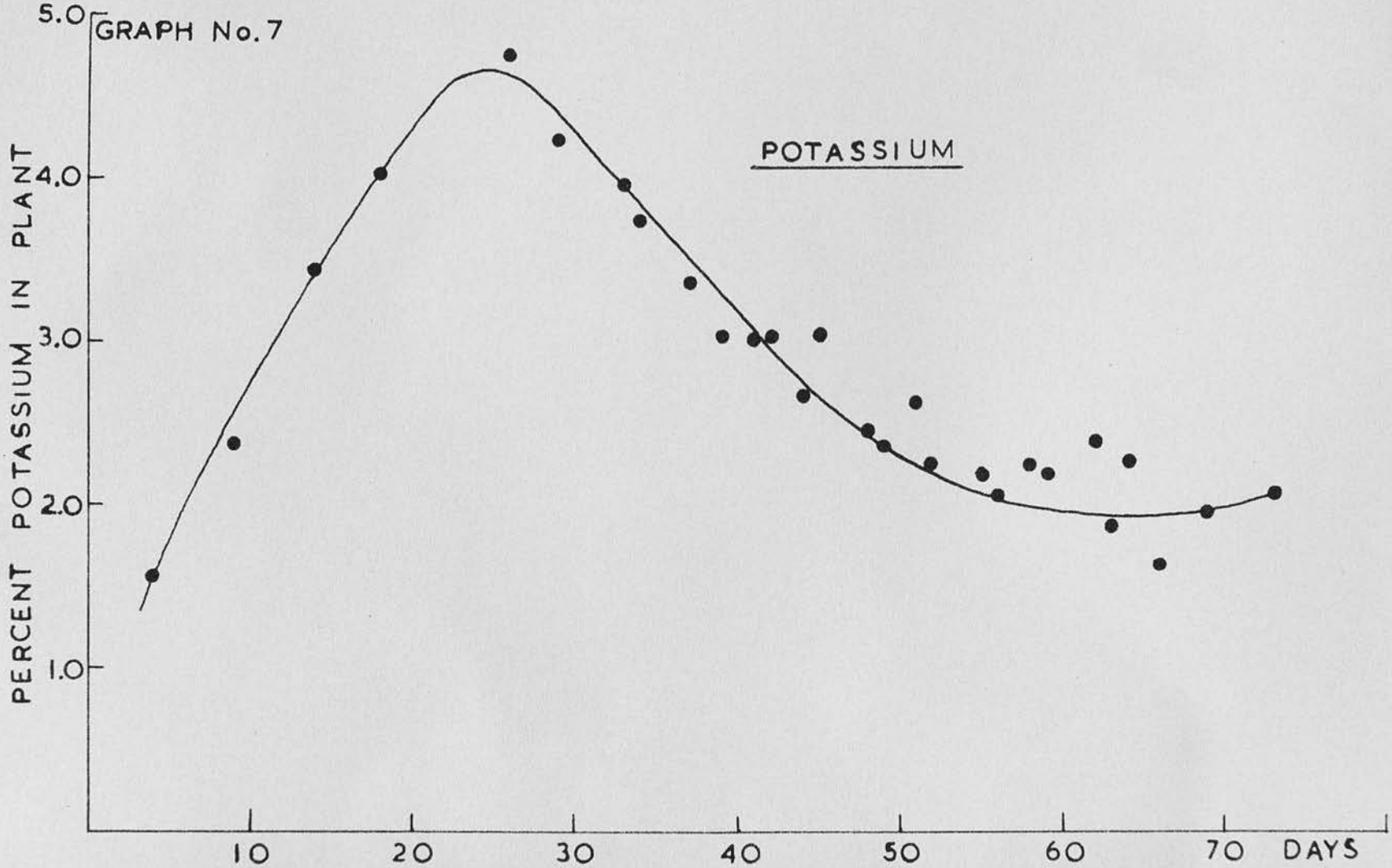
Similarly the change in the magnitude of the nickel-iron ratio in the plant at 40 days, and the accompanying reduction in the level of chlorosis, probably springs from a change in the rate of translocation of nickel relative to iron within the plant. Data given later in this section show that nickel concentrates in the grain of mature plants and therefore even at 40 days was probably being mobilized in the leaves for movement into the developing grain, which emerged at 53 days. This would bring about a reduced nickel-iron ratio in the young leaves which then might not reach the critical level required for the production of chlorosis.

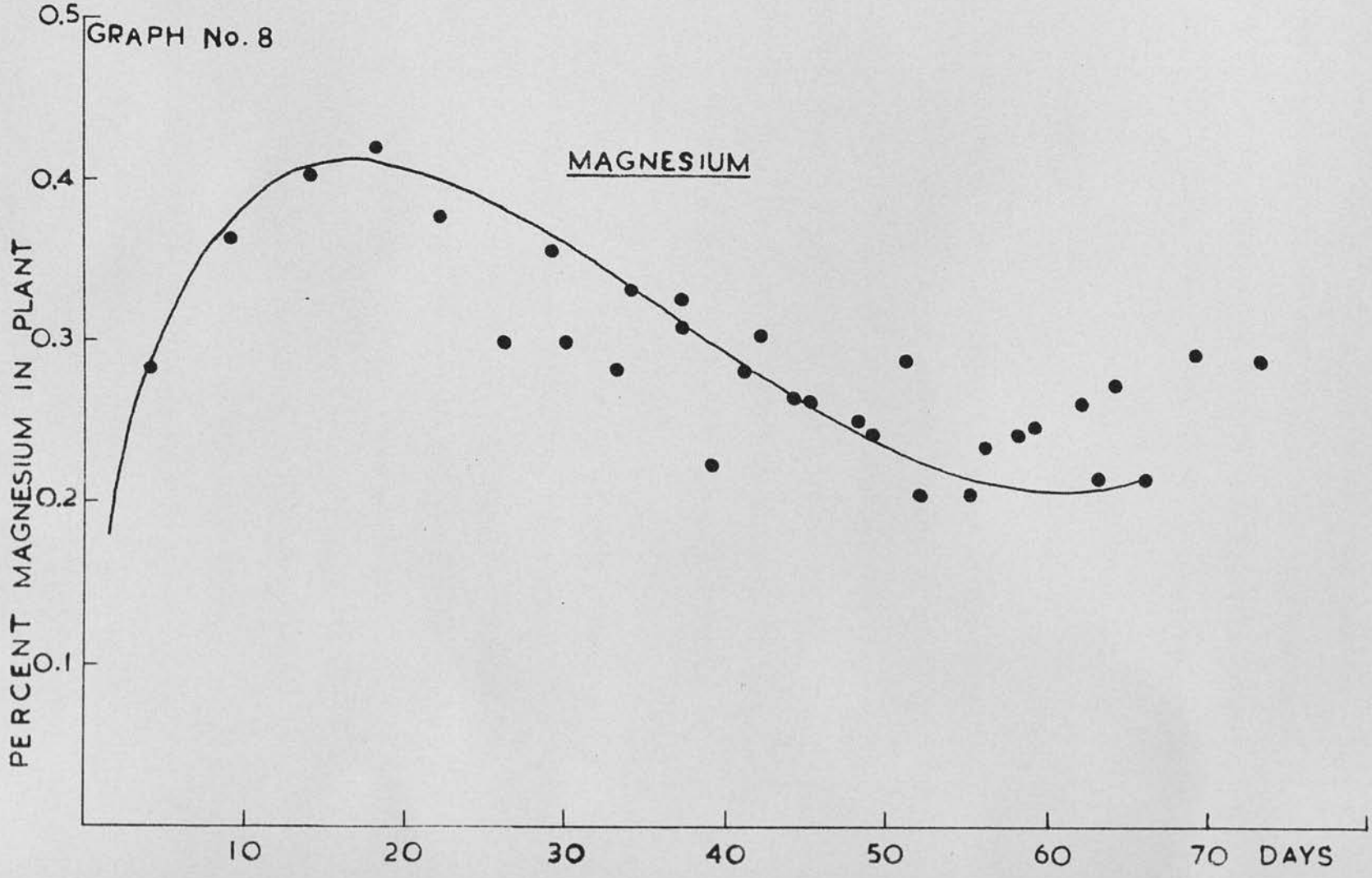
In graph No. 4 when the nickel-iron ratio in the plant was plotted against age of plant, and each chlorosis score* marked/

* only scores of 1 to 6 have been included. Unmarked points are associated with nil symptoms (0).


GRAPH No. 5
PERCENT NITROGEN IN PLANT
5.0
4.0
3.0
2.0
1.0
NITROGEN
10
20
30
40
50
60
70
DAYS


GRAPH No. 6
PHOSPHORUS
PERCENT PHOSPHORUS IN PLANT.
0.7
0.6
0.5
0.4
0.3
0.2
10
20
30
40
50
60
70 DAYS


GRAPH No. 7
POTASSIUM
PERCENT POTASSIUM IN PLANT
5.0
4.0
3.0
2.0
1.0
10
20
30
40
50
60
70
DAYS


GRAPH No. 8
MAGNESIUM
PERCENT MAGNESIUM IN PLANT
0.5
0.4
0.3
0.2
0.1
10
20
30
40
50
60
70
DAYS

marked against the appropriate point, it was seen that the ratio and symptoms increased for about 40 days then decreased for the rest of the experimental period. A quadratic curve was fitted to the data, and gave the following equation.

$$y = -0.0491 + 0.0737x - 0.000868x^2$$

Standard Errors (± 0.0105) (± 0.000129)

where y is the nickel-iron ratio and x the age of the plant in days. The standard errors, with 27 degrees of freedom, of the coefficients of x and x^2 in the equation show the relationship between y and x to be highly significant. When the curve is drawn, the point of inflexion occurs at 41 days, which agrees well with the time at which change in symptom degree was noted.

The distribution of nutrients in mature nickel-toxic oat plants from this experiment was investigated by chemical analysis of grain, leaves and straw. No great differences were found between samples, so the full results for one sample only (taken at 62 days) are given in the table below. The major-element analyses followed the expected pattern in that older tissue generally contained lower amounts of nutrients than young tissue, and stems lower amounts than leaves. The nickel and iron values varied in a similar manner but the highest concentration of both metals was found in the grain. The nickel-iron ratio showed up interesting differences in the distribution of the individual metals. Low ratios were found

in/

in the oldest tissues suggesting that nickel was more readily mobilized and translocated within the plant than iron.

TABLE 19

Distribution of nutrients in mature nickel-toxic oat plants

Plant part	Percentage in dry matter					ppm. in dry matter		Ni/Fe ratio
	N	P	K	Ca	Mg	Fe	Ni	
Grain	2.15	0.290	1.01	0.176	0.154	83	119	1.43
Upper stems	1.72	0.264	1.72	0.165	0.143	48	75	1.56
Upper leaves	3.35	0.235	2.17	1.06	0.410	62	78	1.26
Mid stems	1.45	0.143	2.78	0.297	0.190	37	33	0.89
Mid leaves	1.68	0.146	1.85	0.770	0.335	72	58	0.81
Lower stems	0.94	0.047	1.72	0.102	0.102	22	17	0.77
Lower leaves	1.04	0.110	1.76	1.10	0.433	62	40	0.65

CONCLUSIONS

The beneficial effects seen in the field following the application of lime to the Whitecairns nickel-toxic soils have now been demonstrated in soil-culture experiments. Liming of the soil, either with calcium or sodium carbonate, is found to prevent the appearance of toxicity symptoms and to reduce the uptake of nickel by oat plants. The amount of nickel absorbed is greater at acid pH values than at alkaline values. It is suggested that the reduced uptake of nickel is brought about as a result of a change in the form of the soil nickel, produced by a change in soil pH.

The/

The reduction in uptake of nickel which is produced by liming, is accompanied by a reduction in the level of "exchangeable" nickel in the soil. This effect was studied in a series of laboratory experiments where it was possible to demonstrate that the level of nickel recovered from a limed soil dropped as the soil pH values rose.

While the uptake of nickel from soils falls off with increasing pH, the opposite effect is found on increasing the pH of solution cultures. The absorption of nickel increases with increase in pH, and whilst the intensity of chlorotic symptoms also increases with increase in pH, that of necrotic symptoms is the same irrespective of pH. The implications of this will be discussed more fully in the following section dealing with induced iron deficiency.

These apparently conflicting results serve to emphasise the very great differences existing between soil and solution cultures, in which the factors governing ion absorption are inherently different. The soil is a complex well-buffered system, whereas the nutrient solution is relatively poorly buffered and, unlike the soil, contains no colloidal material which could influence ion absorption. As was mentioned above, the various forms in which nickel exists in soils has not been worked out in any great detail, but Swaine (125) has shown that different amounts of nickel are extracted from soil by using acid or neutral extractants of various types.. What these

results/

results mean in terms of availability to plants is not clear, but it seems probable that the nickel soluble in ammonium acetate represents a fraction available to plants, which is presumably in equilibrium with fractions of successively lower solubility, which are drawn upon to replace nickel removed by plants from the 'available' fraction.

In solution culture, however, it is presumed that nickel salts are fully ionised and that ionic nickel is freely available to plants, although the presence of antagonistic ions in the nutrient solution would modify the amount of nickel absorbed. Change in pH of the solution would also be expected to play a part, due to the changes in concentration of hydrogen and hydroxyl ions. At high pH, for example, a modified form of nickel ion might exist ($NiOH^{+}$) which might be more freely absorbed than Ni^{++}, thus accounting for the enhanced uptake at high pH, which is not wholly due to the reduced availability of iron.

The absorption of nickel and iron by oat plants of different ages was studied in an endeavour to account for changes in symptom degree (particularly chlorosis) seen in the field as plants approached maturity. It was found that while the concentration of iron in the tissues remained relatively constant over a 70-day period, the absorption of nickel increased with time until its concentration in the tissues reached a maximum at about 40 days, thereafter slowly decreasing. The change/

change in the level of nickel in the plant agreed well in point of time with an improvement in the degree of chlorotic symptoms seen in plants of this age.

When the distribution of nickel and iron was determined by analysis of separated plants, it was found that both metals became concentrated in the grain, more in leaves than in stems and more in the young tissue than in old. The mobilization and translocation of nickel would appear to be more readily accomplished than that of iron, since there was a tendency for the latter element to concentrate in the basal portions of the plant.

From the data available it is difficult to explain the observed lack of chlorosis of the last young leaves to be formed. It is not unlikely that the movement of nickel into the grain affects the nickel-iron ratio in the expanding leaf so that the critical ratio for the production of chlorosis is never reached. In this connection, the part played by calcium is not at all clear; nickel-toxic plants absorb calcium to a much greater extent than do healthy plants. Calcium is generally regarded as being quite mobile in the plant and, in view of the effects of calcium found in later sand-culture experiments, its possible participation in this case also, should not be disregarded.

REVIEW OF THE LITERATURE ON IRON DEFICIENCY

Despite the fact that many aspects of iron metabolism in plants have received attention over a lengthy period of time, there are still many points on which information is lacking. The broad outlines of the subject have now been defined, and much of the research has been devoted to a study of the factors causing iron deficiency. There are at least four possible causes of iron deficiency in plants:

(a) a straightforward deficiency caused by a very low iron supply. This is seldom seen in the field.

(b) that seen in crops growing on calcareous soils and usually termed lime-induced chlorosis.

(c) an induced deficiency brought about by excessive concentrations of heavy metals such as nickel, cobalt, copper, manganese, zinc, or chromium.

(d) an induced deficiency produced by a high-level phosphorus supply.

We are not concerned with deficiencies of the first two types in this investigation and they will therefore not be discussed further. The many hundreds of papers listed in the literature of the past 50 years give some idea of the keen interest evinced in induced iron deficiency. These papers fall/

fall into two main groups; firstly, and in the minority, those dealing with chlorosis produced in plants growing on manganiferous soils or soils which have been heavily contaminated by heavy metals e.g. orchard soils which have accumulated copper from fungicidal sprays (1), or as the result of fertilizer practice which recommends small annual dressings of copper salts (109). The remaining, and larger group, describe experiments to investigate this induced iron deficiency under controlled conditions. Here the majority of the papers deal with manganese while the remainder report results obtained with other heavy metals, such as cobalt, copper, zinc and nickel. Manganese has received special attention because it is an essential element (supplied in normal amounts) yet becomes markedly toxic when absorbed in excess. Again, low crop yields in very acid soils can often be attributed to manganese toxicity. Bortner (15) and Berger and Gerloff (12) report toxicity in tobacco and potatoes respectively.

Early work with pineapple in Hawaii showed that iron applications to the soil would cure the chlorosis induced by the high levels of soil manganese, (Martin (80)). Other workers advocated heavy phosphate dressings and reported increased yields and reduced symptoms (Kelley (63)). This antidoting effect of iron led to solution-culture work which confirmed that an increase in iron supply could inhibit the production of chlorosis (Hopkins, Pagan & Ramirez-Silva (51)).

These/

These early papers on heavy metal toxicities confined themselves to describing symptoms in detail and to discussing possible remedial measures. More recently, however, attempts have been made to explain how the individual heavy metals interfere with iron metabolism.

The form and function of iron in the plant have been discussed by several authors, and Lindner and Harley (70) have summarised the state of knowledge in 1944. Iron is associated largely with cell nuclei and chloroplasts; it is mainly absorbed on phosphorus-containing proteins(Noack and Liebich (100)) and is a constituent of several porphyrin enzymes. As a catalyst in chlorophyll formation, the iron-containing chlorophyll enzyme is among the least stable of iron compounds and thus is the one most likely to be affected by any metabolic upset, resulting in the appearance of chlorosis in the plant.

The total-iron content of plant tissue is generally considered to be of little value in assessing the iron status of plants, because iron deficiency usually results from a lack of available iron within the tissues rather than from an insufficient supply from the substrate (Goodall and Gregory (31)). In chlorotic tissue the total-iron content is frequently greater than that of normal tissue. This has been recognised for some time (for example Milad (85): Roach (110); Wallace and Hewitt (140): Millikan (88): Nicholas (97)): but Thorne and Wallace (126) consider that the total-iron content/

content may be significant if measured on the basis of leaf area rather than dry-matter. Some workers prefer to base their estimate of iron status on the water-soluble iron fraction(Nicholas (97)) or on the iron extracted by 0.5 N HCl (Lindner and Harley (70)) or on the iron content of the expressed sap (Rogers and Shive (113)).

The literature, then, provides ample confirmation that iron deficiency is generally caused not by an insufficiency of iron in the plant but by the unavailability of the iron present. It is to be noted, however, that Kriel (65) reports a reduction in total-iron content produced by excess manganese, while Sideris (120) found that translocation of iron from roots to leaves was considerably reduced by the presence of excessive manganese. Vergnano's analytical results show that nickel also reduced the total iron content. Confirmation of her findings will be given in experimental results listed later in this section.

Several theories have been put forward to explain non-utilization of iron in induced iron deficiency. Rogers and Shive (113) report reduction in iron solubility due to relatively high sap pH, but Bennett (11) has shown that the sap of iron-deficient leaves does not necessarily have a higher pH than that of normal leaves. Somers and Shive (123) suggest that as the active iron is in the ferrous state (Kliman (64): Shive (119): Thorne and Wallace (126): Hewitt (44)) the mechanism by which excess manganese induces iron/

iron deficiency is the oxidation of ferrous iron (by Mn^{++++}) to ferric iron which is then precipitated as unavailable ferric phosphorus organic complexes. The basis of this argument is that the oxidation-reduction potential of manganese ($Mn^{++++} \longrightarrow Mn^{++}$) is greater than that of iron, but Hewitt (41, 42) points out that this theory breaks down when extended to other trace-element toxicities and, though more plausible if associated with the redox potentials of complex ions, it still does not cover all the facts. In any case, Leeper (67) emphasises that the original hypothesis is fallacious since Mn^{++++} could not exist in plant tissues without causing irreparable damage.

A more probable explanation is that the toxic metal replaces iron in some organic complex thus preventing some essential function; Baudisch (9) points out that trace-element antagonism may be explained in the replacement of one trace element by another in an organic complex; Jacobson (60) found iron to be associated with a chlorophyll protein complex, while Bennett (11) contends that if iron is a constituent of such a complex it would be the so-called "active" iron. If such a complex exists Bennett's results showed that its iron content was not constant during the growing season, from which he concluded that the active iron was acting as a catalyst rather than merely a constituent of a complex.

Sideris/

Sideris (120) presents evidence to show that iron occurs in combination with a proteinaceous substance as an enzyme and is indirectly associated with chlorophyll; Sideris and Young (122) suggest that manganese-induced iron deficiency is brought about by the replacement of iron by manganese in a chlorophyll precursor, but Hewitt (44) considers that replacement of magnesium by manganese is more probable. In the same article Hewitt draws attention to the order of stability of metal-organic complexes (Mellor and Maley (83, 84); Irving and Williams (59); Maley and Mellor (79)), and of metal-porphyrin complexes (Ganick and Gilder (29), and Lemberg and Legge (68)), and points out the similarity between these and that of trace-element ability to induce iron deficiency. Hunter and Vergnano (57), as mentioned previously, examined the toxic action of several heavy metals and arranged them in an order based on their ability to produce toxicity in oats; an order which agreed well with those mentioned above. Although this theory is the most acceptable of those proposed, Hewitt (41, 42) emphasises that at present there is no definite proof that all trace elements induce chlorosis in the same way. Again, although trace-element toxicities can be related to induced iron deficiency, the fact that each different heavy metal produces a different specific toxic effect in addition to chlorosis, serves as a warning against an over-simplified treatment of the subject.

Many/

Many authors regard the iron-manganese ratio in plants as an important aid in the interpretation of results (see, for example: Tottingham and Beck (129); Scharrer and Schropp (118); Shive (119); Twyman (130)). Others, such as Hopkins, Pagan and Silva (51), while admitting the usefulness of the concept, contend that the total concentration present must always be considered. Stronger criticism of the iron-manganese ratio is made by Bennett (11); Wallace and Hewitt (140); Millikan (89); Nicholas (96) and Hewitt (44). In her solution-culture work Vergnano made use of the nickel-iron ratio in the plant and showed that a low ratio was associated with a low degree of necrosis, but that, in general, the experimental results could be explained without recourse to the ratio.

A further factor in iron deficiency which must now be considered in view of its possible effects on nickel toxicity, is the effect of phosphorus on the uptake and translocation of iron. The subject is a vexed one; many papers support the contention that a high level phosphorus supply will induce chlorosis while almost as many refute this claim.

A common feature of induced iron deficiency is the relatively high phosphorus content of the chlorotic leaves (see Olsen (102); Chandler and Scarseth (17); Lindner and Harley (70)) which is consistent with the theory of Somers and Shive (123) than iron is finally made unavailable in the plant/

plant by union with a phosphorus organic complex. Such precipitation has been demonstrated by chemical tissue tests carried out by Olsen (102) who showed that much of the iron was immobilized in the veins of chlorotic leaves.

The effect may also to some extent be external, since Chapman (19); Chapman, Liebig and Vanselow (20) and Millikan (89) have shown that chlorosis can be produced in plants grown in nutrient solutions high in phosphorus. Similar effects have been seen following high phosphorus applications to certain soils (Sideris and Krauss (121); Chandler and Scarseth (17); Walsh and Clarke (141); and Wallace and Hewitt (140)). In view of the results for nickel to be given later, Millikan's (89) report that increased phosphorus in the nutrient solution accentuated the chlorotic symptoms produced by excess manganese, is doubly interesting. Chandler and Scarseth (17) and Biddulph (13) further state that high-level phosphorus actually reduces the total-iron content of the leaves in addition to decreasing its internal availability.

While most authors regard the phosphorus-iron effect as occurring within the plant, Franco and Loomis (27) consider that their solution-culture experiments prove the effect to be wholely external and caused by soluble phosphate at pH 6 and over.

In direct contrast, Milad (85) and Bennett (11) point out that high concentrations of phosphorus in the plant are not always associated with iron deficiency, and furthermore, the second/

second author has shown that iron deficiency chlorosis is not produced when the phosphorus content of the leaf is considerably increased by injection. Bennett then goes on to propose that the critical factor may be the relative rate of supply of iron and the rate of absorption of iron by "residual" protein; if this theory is correct, this rate of absorption would probably be greater the higher the concentration of residual protein, and consequently, any increase in residual protein such as might be brought about by an increased phosphorus supply, would lead to a reduction in the leaf content of iron, deficiency being the more quickly produced if the rate of supply of iron was also low.

From the foregoing, it would appear that the rate of supply of phosphorus relative to iron is a factor which should be borne in mind in dealing with problems of iron deficiency. The analytical results obtained by Vergnano (132) in her pH experiment show that the nickel-toxic plants had a consistently higher phosphorus content than their partners grown at the same pH. This could be the relatively high concentration of phosphorus found in chlorotic plants as reported in the literature or it could be an accumulation of phosphorus resulting from the toxic action of nickel on the general metabolism of the plants.

Other aspects of the nickel-iron relationship investigated by Vergnano showed that the level of iron supply was an important factor in determining the degree of toxic symptoms which

would be produced. Nickel toxicity symptoms (both necrosis and chlorosis) in sand-cultured oats were found to be less severe when the concentration of iron in the nutrient solution was high. An inverse relationship was found to exist between the nickel and iron contents of the plants; the nickel content was materially reduced by high concentrations of iron in the nutrient solution, and the iron content by nickel, the former being the more pronounced effect.

In anatomical studies of nickel-toxic and iron-deficient oat tissue, Vergnano and Hunter (134) had suggested that necrosis might be no more than the result of severe iron deficiency. This possibility was not borne out by the results of the experiment described above, a high-level iron supply reducing the severity of necrosis no more than could be accounted for the reduction in nickel content. Considered together, Vergnano's results do not make it clear whether this nickel-iron antagonism occurs wholly within the plant or is a substrate phenomenon. Solution-culture experiments now to be described were designed with a view to extending our information on this point.

STUDY OF THE NICKEL - IRON RELATIONSHIP

Effect of pH and iron level on uptake of nickel

The complexed form of iron was used at two levels (1.2 and 12 ppm.) and uptake of nickel was studied in two pH ranges (4.0 - 4.5 and 6.0 - 6.5). Nickel, as in most solution-culture experiments, was supplied at 1.2 ppm., a level sufficiently toxic to give symptoms of medium intensity. There were two crocks per treatment and solutions were changed weekly. The experiment ran for 21 days. The concentrations of nickel and iron found in the laminae are given in the table below together with the symptoms and yield of fresh material recorded at harvest.

TABLE 20

Nickel and iron absorption at pH 4.25 and 6.25

Treatment	pH	Yield per 2 pots (g.)	Concentration in laminae Fe (ppm.)	Ni	Toxic symptoms Necrosis	Chlorosis
low Fe:0-Ni	4.25	76.4	74	--	-	-
low Fe + Ni	4.25	69.0	56	81	M	L
high Fe:0-Ni	4.25	68.8	81	--	-	-
high Fe + Ni	4.25	70.6	58	53	0	0
low Fe:0-Ni	6.25	85.5	62	--	-	-
low Fe + Ni	6.25	46.9	38	138	VH	H
high Fe:0-Ni	6.25	86.1	81	--	-	-
high Fe + Ni	6.25	72.8	64	64	0	0

The/

The results provided confirmation of the beneficial effect of a high iron supply in preventing the appearance of toxicity symptoms, although the uptake of nickel by these plants was still appreciable, and greater at the higher pH than at the lower. The same effect of pH held for the low-iron cultures, plants grown at the higher pH containing more nickel and having more severe symptoms. The iron analyses showed that in every case nickel had reduced the uptake of iron. In the absence of nickel, uptake of iron was greater at low pH in the case of low-iron plants, whereas pH had not affected the uptake from the high-level supply of iron.

Examination of the roots of these cultures showed interesting differences produced both by variation in pH and rate of iron supply. The effect of nickel, in general, was to inhibit the production of secondary rootlets. The roots most affected were those in the low-iron culture at pH 6.25, where the root length was much reduced (18 cm. as against 30 cm. in the corresponding control) and the overall effect was of a brownish straggling root system with very few secondary roots, which were confined to the top half of the system. A "browning" of the roots which had been noted before, was found not to be confined to +nickel-cultures but occurred also where iron supply was high. Previously it had been thought that one of the toxic effects of nickel might be to immobilise iron in or on the roots. No evidence of such an effect could be obtained here but the subject will be dealt with more fully in a later section where root analysis was used in an attempt to provide information on this point.

Uptake/

Uptake of nickel with varying pH in the absence of iron

In these experiments to study the effect of pH on the uptake of nickel, the presence of iron in the nutrient solution must have played a part in affecting the final results obtained. It was felt that solubility differences, not to mention possible effects resulting from the different forms of iron used, could be affecting nickel absorption. It was decided therefore to grow plants at optimum pH (say 5.5) with either ferric citrate or Fe-EDTA (at 1.2 ppm.) as iron source for about 7 - 10 days and then to transfer them to solutions containing 1.2 ppm. nickel (but no iron) held at pH of 4, 5, 6 and 7, and allow growth to proceed for a further 7 - 10 days. A set of control plants in solutions containing no iron and no nickel would be used to check that iron deficiency per se did not appear during the course of the experiment. If none appeared in these controls then chlorosis would be due solely to the direct toxic effect of nickel.

Oat plants (20 - 22 cm. tall) were transferred to the solutions of varying pH after seven days growth in the solutions of pH 5.5. Necrosis appeared first at pH 4 and 5 after three days, while plants at pH 6 and 7 were still normal although slightly chlorotic. At the end of eight days when the plants were harvested, all treatments showed chlorosis (although no chlorosis had appeared in the control treatments) increasing in severity with increasing pH. Necrosis, of moderate severity, was present at each pH level and affected the mid-

thirds/

thirds of basal and intermediate leaves and the tips of young leaves. The way in which symptoms developed suggested that there was no movement of iron from the old into the young leaves, since at harvest basal leaves were still green although having necrotic areas.

The analytical results for the nickel treatments of both iron series are set out below.

TABLE 21

Absorption of nickel in pH range 4 - 7.

	Fe-EDTA series			Ferric citrate series		
Culture pH	Fresh Yield per 2 pots (g.)	Concentration in laminae (ppm.) Ni	Fe	Fresh Yield per 2 pots (g.)	Concentration in laminae (ppm.) Ni	Fe
4	85.9	63	70 (89)*	94.9	64	67 (93)*
5	83.7	106	57 (94)	84.3	102	48 (99)
6	72.6	130	55 (87)	76.8	113	48 (85)
7	62.3	127	62 (91)	85.1	118	60 (90)

* Iron analyses for control plants are given in brackets.

Once again nickel uptake has been shown to increase with pH and on this occasion the effect is independent of iron supply. The results for iron in the nickel-toxic plant material are surprising and suggest, when compared with controls, that these figures represent the actual iron present in the plants at the time of transfer to the solutions containing nickel. The iron already in the roots of these plants was immobilized there by the nickel, whereas in the controls it was free/

free to move into the tops thus accounting for the higher levels found in the laminae of the control plants. The possibility that nickel damaged the root tissues so that there was a loss of iron to the nutrient solution must also be considered.

In the course of these experiments it was noticed that there appeared to be an intensification of toxic symptoms, particularly chlorosis, due to high light intensities or increased temperature. The crocks were set out on trolleys lying parallel with, and close to, the greenhouse wall. The two crocks in each treatment were arranged in pairs, one in front of the other so that one crock in each pair was closer to the heating pipes and side wall of the greenhouse. Plants in these crocks always seemed slightly more severely affected than their partners. Reference to the literature showed that this type of effect had been noted before in manganese-induced iron deficiency. McCool (78) studied the effect of light intensity on the degree of toxic symptoms produced in buckwheat (and other plants) and found that the visible injury and uptake of manganese decreased as the light intensity decreased. More recently, Löhnis (75) comments on a temperature effect which intensified manganese toxicity symptoms in beans grown in solution-culture in a greenhouse.

There were indications by this stage that the main factor governing the uptake of nickel (and the toxic symptoms produced) was the relative proportions of nickel and iron in the substrate.

A/

A preliminary experiment (not reported here) in which plants were grown first on either low or high iron supply showed that when these plants were transferred to solutions containing nickel but no iron, both sets of plants were affected just as quickly. There appeared to be no benefit gained from a high initial concentration of iron in the tissues.

Uptake of nickel in presence of different iron levels in the nutrient solutions

In this experiment plants were grown on a normal (1.2 ppm.) iron supply (as Fe-EDTA) at pH 5.5 for 7 days before being transferred to solutions of pH 4 containing no iron, 1.2 ppm. iron or 12 ppm. iron respectively. A further treatment involving high iron at pH 6 was also used. The plants were grown under these conditions for a further 10 days before being harvested. The symptoms noted at that time and the level of nickel found in the leaf dry-matter are given below.

T A B L E/

TABLE 22

Effect of iron level on absorption of nickel

Nutrient solution composition	Fresh weight yield/100 plants (g.)	Concentration in dry matter Fe (ppm.)	Ni	Toxic symptoms Necrosis	Chlorosis
pH 4 0-Fe	52.8	104	--	-	L-
4 0-Fe + Ni	49.4	100	130	M	M
4 Normal Fe	63.9	148	--	-	-
4 Normal Fe + Ni	52.9	105	98	L+	L
4 High Fe	53.5	119	--	-	-
4 High Fe + Ni	46.0	103	63	-	-
6 High Fe	60.9	101	--	-	-
6 High Fe + Ni	48.0	84	85	M+	M+

The figures above confirm the view that the relative concentrations of iron and nickel in the substrate determine the degree of toxic symptoms and uptake of nickel which will result (other things being equal). It will be noted that nickel uptake at pH 6 was greater than at pH 4 for high iron cultures and that at pH 6 the level of iron absorbed was not high enough to prevent the appearance of toxic symptoms. A set of experiments described below, also in solution-culture, involving wide variation in the proportions of nickel and iron in the nutrient solution, provided further evidence to support the above contention. Lastly, a 'split-root' experiment with tomato demonstrated very clearly the external nature of the nickel-iron antagonism although there were indications at a

certain/

certain stage of growth of the existence of an internal antagonism.

Effect of substrate nickel-iron ratio on the production of toxic symptoms.

Three separate experiments of this type were carried out; they will be described individually and the analyses given, and then the results will be considered together and the conclusions presented. The chelate form of iron was used throughout this series and the pH of the nutrient solution used was maintained at 5.0. Each experiment ran for 12 days and solutions were changed every 4 days. At harvest, yields of the three crocks per treatment were combined, dried whole, and major elements, nickel, and iron determined in the dry-matter. Root analysis was attempted for the first time in this investigation, although no attempt was made to differentiate between nutrients on the roots, as opposed to in the roots. Some difficulty was experienced in determining nickel in the root extracts because of interference due to their high iron content. Eventually it was found easiest to remove most of the iron by shaking up an aliquot of the extract (made 6 N by the addition of concentrated hydrochloric acid) with successive volumes of ether. The iron was removed in the ether layer leaving the nickel (and some iron) in the aqueous layer which was then evaporated to dryness and taken up in 20 ml. 5% hydrochloric acid, filtered, and diluted to 100 ml. with distilled water.

The/

The method used was substantially that of Piper (105) as modified by Hewitt (45).

The first two experiments between them covered nickel-iron ratios of 0.1, 0.2, 1.0, 2.0, 10.0 and 20.0 over three concentration ranges in each case. Details of the nickel and iron contents of the nutrient solutions are given below.

TABLE 23

Substrate nickel-iron ratios

Experiment 1				Experiment 2			
Treat. No.	ppm. in solution Ni	Fe	Ni/Fe	Treat. No	ppm. in solution Ni	Fe	Ni/Fe
750	0.12	0.12	1.0	767	0.12	0.06	2.0
751		1.20	0.1	768		0.60	0.2
752	1.20	0.12	10.0	769	0.48	0.024	20.0
753		1.20	1.0	770		0.24	2.0
754		12.0	0.1	771		2.4	0.2
755	2.40	0.24	10.0	772	1.92	0.096	20.0
756		2.4	1.0	773		0.96	2.0
757		24.0	0.1	774		9.6	0.2

Necrotic symptoms appeared in the first experiment after two days and affected plants growing in solutions having the highest nickel-iron ratio and highest concentration of nickel in the solution. At this time all plants in solution of ratio 0.1 were healthy irrespective of nickel concentration in the solution. However, at the end of a further two days, only

plants/

plants of Treatment 751 were still healthy; necrosis having appeared in Treatments 754 and 757 which had the same ratio but higher absolute amounts of nickel present. After eight days chlorosis appeared- Treatments 752 and 755 were moderately chlorotic, 753 and 756 slightly chlorotic, while the remaining treatments were not affedted. At harvest it was evident that for the same nickel-iron ratio in the solution symptoms were worst in the plants grown in solutions containing most nickel. That is brought out in the table below.

TABLE 24

Effect of solution Ni/Fe ratio on absorption of nickel and iron

Treat. No.	Solution Ni/Fe ratio	Total yield dry matter	Weight of dry roots	Tops (ppm.) Iron	Tops (ppm.) Nickel	Roots (%) Iron	Roots (ppm.) Nickel	Necrotic Symptoms
750	1.0	6.28	2.31	75	59	0.11	253	0
751	0.1	6.01	2.28	83	41	0.32	116	0
752	10.0	5.24	1.94	48	116	0.13	1319	M+
753	1.0	5.85	2.34	51	100	0.32	928	M
754	0.1	6.11	2.67	62	54	1.08	178	0
755	10.0	3.91	1.44	49	141	0.25	1805	VH
756	1.0	3.45	1.37	50	113	0.81	742	H+
757	0.1	5.98	2.98	79	56	2.42	299	L-

The figures in Table 24 also confirm the "see-saw" nature of the nickel-iron uptake picture*. Where one is high, the other is low and vice versa.

* to which Vergnano first directed attention.

At each level of nickel supplied (0.12, 1.2 or 2.4 ppm.), increase in iron level is found to reduce the concentration of nickel in tops and roots, and is accompanied by slight increases in the iron content of the tops, and marked increases in that of the roots. The percentage translocation (Table 25) of iron is found to be lower than that of nickel from nutrient solutions containing the two elements in equal amounts. As the absolute amounts of each element increase, the percentage translocation of iron falls off very rapidly (15.6, 3.9, 1.5) while that of nickel varies only slightly (38.9, 21.3, 27.6).

TABLE 25

Total uptake of nickel and iron

Treat. No.	Iron (mg.) Tops	Iron (mg.) Roots	Nickel (mg.) Tops	Nickel (mg.) Roots	Total content (mg.) Iron	Total content (mg.) Nickel	% translocated Iron	% translocated Nickel	Necrotic Symptoms
750	0.47	2.54	0.37	0.58	3.01	0.95	15.6	38.9	0
751	0.50	7.29	0.25	0.26	7.79	0.51	6.4	49.0	0
752	0.25	2.52	0.61	2.56	2.77	3.17	9.0	19.2	M+
753	0.30	7.49	0.59	2.17	7.79	2.76	3.9	21.3	M
754	0.38	28.84	0.33	0.48	29.22	0.81	1.3	40.7	0
755	0.19	3.60	0.55	2.60	3.79	3.15	5.0	17.4	VH
756	0.17	11.10	0.39	1.02	11.27	1.41	1.5	27.6	H+
757	0.47	72.12	0.33	0.89	72.59	1.22	0.6	27.1	L-

The symptoms recorded in the second experiment followed the pattern of the first, in that the plants to become necrotic first were those in solutions containing the highest level of nickel and having the widest nickel-iron ratio. After four days, treatments 772 and 773 had necrotic symptoms which increased in intensity during the next few days. After seven days, plants in treatment 774 first showed necrosis which spread rapidly until all plants in the crock were affected. By this time slight symptoms had appeared in treatments 769 and 770. At harvest the position was :-

TABLE 26

Treatment	Ni/Fe ratio	Necrosis	Chlorosis
767	2.0	0	0
768	0.2	0	0
769	20.0	L+	L+
770	2.0	L	L
771	0.2	0	0
772	20.0	VH	VH
773	2.0	H	H+
774	0.2	M-	M-

Comparison with Table 24 shows that necrotic symptoms are milder which is in keeping with the lower levels of nickel used in this experiment, although the fact that the nickel-iron ratios here are higher might have been expected to produce/

produce more severe symptoms. The inference must be that concentration of nickel in the substrate plays a greater part in determining toxicity than does the nickel-iron ratio. The yields and analytical data are set out in Tables 27 and 28.

TABLE 27

Effect of solution Ni/Fe ratio on absorption of nickel and iron

Treat.	Solution Ni/Fe ratio	Total yield dry matter (g.)	Weight of dry roots (g.)	Tops (ppm.) Iron	Tops (ppm.) Nickel	Roots (%) Iron	Roots (ppm.) Nickel	Necrotic Symptoms
767	2.0	5.72	2.17	76	44	0.12	354	0
768	0.2	5.85	1.91	77	37	0.35	117	0
769	20.0	5.77	2.18	67	79	0.07	1087	L+
770	2.0	5.79	2.22	68	70	0.16	739	L
771	0.2	6.11	2.61	79	57	0.43	374	0
772	20.0	3.27	1.45	45	112	0.17	669	VH
773	2.0	3.92	1.69	64	118	0.50	834	H
774	0.2	5.96	2.34	104	86	0.82	624	M-

TABLE

TABLE 28

Total uptake of nickel and iron.

Treat. No.	Iron (mg.) Tops	Roots	Nickel (mg.) Tops	Roots	Total content (mg.) Iron	Nickel	% translocated Iron	Nickel	Necrotic Symptoms
767	0.43	2.60	0.25	0.77	3.03	1.02	14.1	24.5	0
768	0.45	6.69	0.22	0.22	7.14	0.44	6.3	50.0	0
769	0.39	1.53	0.46	2.37	1.92	2.83	20.3	16.2	L+
770	0.39	3.55	0.41	1.64	3.94	2.05	9.9	20.0	L
771	0.48	11.22	0.35	0.98	11.70	1.33	4.1	26.3	0
772	0.15	2.47	0.37	0.97	2.62	1.34	5.7	27.6	VH
773	0.25	8.45	0.46	1.41	8.70	1.87	2.9	24.5	H
774	0.62	19.19	0.51	1.46	19.81	1.97	3.1	25.8	M-

In the third experiment the nickel concentration was held constant at 1.2 ppm. while iron was varied from 0.12 to 12.0 ppm. in the nutrient solution to produce a range of nickel-iron ratios varying between 0.1 and 10.0. The high amounts of iron found in the roots in the two earlier experiments showed the need for iron analyses of normal roots. This could conveniently be brought in at this stage by growing oat plants in a series of solutions of increasing iron content which were, in fact, the same iron levels as were being used in the third experiment now under discussion. It would thus be possible to compare the iron content of roots and tops of normal and nickel-toxic plants and ascertain how nickel was affecting the absorption and translocation of iron.

TABLE 29

Effect of substrate Ni/Fe ratio on absorption of nickel and iron.

Treat. No.	Ni/Fe ratio in the nutrient solution	Total Yield (g.) of dry matter	Weight (g.) dry roots	Tops (ppm.) Iron	Tops (ppm.) Nickel	Roots (%) Iron	Roots (ppm.) Nickel	Necrotic Symptoms
775	10.0	3.13	1.28	61	64	0.22	484	M
776	2.0	3.43	1.35	53	64	0.32	471	M
777	1.33	2.67	1.06	47	61	0.50	491	M
778	1.00	3.04	1.22	53	68	0.56	511	M+
779	0.40	2.99	1.25	58	49	0.97	422	L+
780	0.20	3.07	1.27	52	50	0.92	300	L
781	0.13	3.32	1.41	55	41	1.12	255	L
782	0.10	3.31	1.37	57	37	1.18	216	L-

TABLE 30

Total Uptake of nickel and iron

Treat. No.	Iron (mg.) Tops	Iron (mg.) Roots	Nickel (mg.) Tops	Nickel (mg.) Roots	Total content Iron	Total content Nickel	% translocated Iron	% translocated Nickel	Necrotic Symptoms
775	0.19	2.82	0.20	0.62	3.01	0.82	6.3	24.4	M
776	0.18	4.32	0.22	0.64	4.50	0.86	4.0	25.6	M
777	0.13	5.30	0.16	0.52	5.43	0.68	2.4	23.5	M
778	0.16	6.83	0.21	0.62	6.99	0.83	2.3	25.3	M+
779	0.17	12.13	0.15	0.53	12.30	0.68	1.4	22.1	L+
780	0.16	11.68	0.15	0.38	11.84	0.53	1.4	28.3	L
781	0.18	15.79	0.14	0.36	15.97	0.50	1.1	28.0	L
782	0.19	16.17	0.12	0.30	16.36	0.42	1.2	28.6	L-

Table 31 below shows clearly that the concentration of iron in the tops of the plants was remarkably constant and thus practically independent of the level of iron in the nutrient solution. The roots on the other hand accumulated iron at a rate consistent with the level of iron supplied. Even at the lowest level of iron supply, where accumulation in the roots was smallest, the percentage of iron which found its way to the upper parts of the plant was surprisingly small (5.1%). At the other end of the scale the percentage translocated was only 0.8. When these iron analyses were compared with those obtained from plants grown in solutions with 1.2 ppm. nickel supplied (Tables 29 and 30) it was seen that nickel has consistently reduced both the concentration and/

TABLE 31

Absorption of iron from solutions of differing iron content

Treat. No.	level of iron in nutrient solution (ppm.)	Total yield of dry matter (g.)	Weight dry roots (g.)	Concentration (ppm.) Tops	Concentration (%) Roots	Total Uptake (mg.) Tops	Total Uptake (mg.) Roots	Total Content (mg.)	% translocated
811	0.12	4.34	1.78	57	0.26	0.25	4.63	4.88	5.1
812	0.60	4.45	1.74	60	0.37	0.27	4.83	5.10	5.3
813	0.90	4.24	1.64	66	0.40	0.28	6.56	6.84	4.1
814	1.20	4.48	1.55	60	0.34	0.27	5.27	5.54	4.9
815	3.0	4.15	1.88	60	0.86	0.25	16.17	16.42	1.5
816	6.0	3.73	1.50	62	1.03	0.23	15.45	15.68	1.5
817	9.0	4.05	1.87	62	1.35	0.25	25.25	25.50	1.0
818	12.0	3.39	1.70	64	1.65	0.22	28.05	28.27	0.8

and total amount of iron found in the plants. The percentage translocation of iron was also reduced at each nickel-iron ratio; the effect being particularly marked for ratio values of 1.0 and 1.33 (where incidently, the nickel content of roots and tops was rather higher than was to be expected from examination of the results for the other ratios).

The variations in iron and nickel supply used in these three experiments were found to have only slight effects on the major-element composition of the plants. The levels of phosphorus, potassium, calcium, and magnesium found in plants from the third experiment are given below:-

TABLE 32

Concentration of major elements in dry matter (%)

Solution (ppm.) Iron level	Phosphorus		Potassium		Calcium		Magnesium	
	0-Ni	+Ni	0-Ni	+Ni	0-Ni	+Ni	0-Ni	+Ni
0.12	1.20	1.58	7.43	6.43	0.454	1.019	0.235	0.282
0.60	1.25	1.47	7.25	7.01	0.432	0.959	0.234	0.311
0.90	1.25	1.43	7.37	6.88	0.425	1.131	0.234	0.274
1.20	1.28	1.42	7.20	7.06	0.424	1.010	0.232	0.261
3.00	1.35	1.33	7.29	7.31	0.393	1.027	0.205	0.269
6.00	1.48	1.37	6.90	7.17	0.424	1.000	0.235	0.274
9.00	1.56	1.33	7.10	7.24	0.427	0.991	0.231	0.240
12.00	1.56	1.39	6.86	6.65	0.425	0.897	0.237	0.261

The/

The concentration of calcium in the dry-matter was more than doubled in the presence of nickel. The magnesium content was similarly affected, only in this case, the increase was slight. These effects will be discussed more fully in a subsequent section dealing with the effect of nickel on the absorption of major nutrients. The phosphorus figures showed certain abnormal features, nickel apparently increasing the concentration of phosphorus at low iron levels and reducing it at high iron levels. An mentioned before, Vergnano comments on this apparent accumulation of phosphorus in the tissues but states that while it may be a factor in nickel toxicity, it has to be considered along with other factors.

TABLE 33

Concentration of major elements in roots (%)

Solution (ppm.) Iron level	Phosphorus		Potassium		Calcium		Magnesium	
	0-Ni	+Ni	0-Ni	+Ni	0-Ni	+Ni	0-Ni	+Ni
0.12	0.97	1.27	3.80	5.55	0.20	1.22	0.99	0.79
0.60	1.04	1.21	3.65	5.63	0.17	1.16	1.07	0.79
0.90	1.09	1.27	3.88	5.33	0.19	1.53	1.05	0.73
1.20	1.03	1.30	3.46	5.66	0.17	1.28	1.00	0.73
3.00	1.24	1.48	3.17	5.44	0.20	1.26	0.94	0.87
6.00	1.52	--	3.69	--	0.22	--	1.04	--*
9.00	1.45	1.79	3.12	4.82	0.23	1.15	0.96	0.95
12.00	1.53	1.93	3.46	4.78	0.29	1.20	0.88	0.93

* extract lost.

When/

When the major element content of the roots was similarly examined (see table 33) nickel was seen to have increased the concentration of phosphorus, potassium and calcium but decreased that of magnesium. Since phosphorus in the roots is consistently higher in the roots of nickel-toxic plants, irrespective of iron supply, yet varies in the tops as noted above, it would seem that at low iron levels phosphorus moves freely into the tops whereas at high iron levels much of that absorbed remains in the roots. It is not possible to say whether this effect is due to iron or nickel, although the former is more likely to be involved. Since potassium in the tops is not clearly affected by iron or nickel supply and tends to be increased in the roots in the presence of nickel, it would appear that nickel, and to a certain extent iron at high level, is affecting its movement into the tops.

The anomalous position of calcium with respect to its uptake and effect on toxic symptoms will be discussed more fully in a later section (see p. 153). The results here confirm those obtained in later experiments, and show that the concentration of calcium is consistently increased in both roots and tops by nickel. Magnesium, on the other hand, is descreased in the roots in the presence of nickel, although some slight increase in concentration in the tops of nickel-toxic plants is noted.

As a result of her experiments on the iron-nickel relationship, Vergnano (132) showed that the degree of necrotic symptoms/

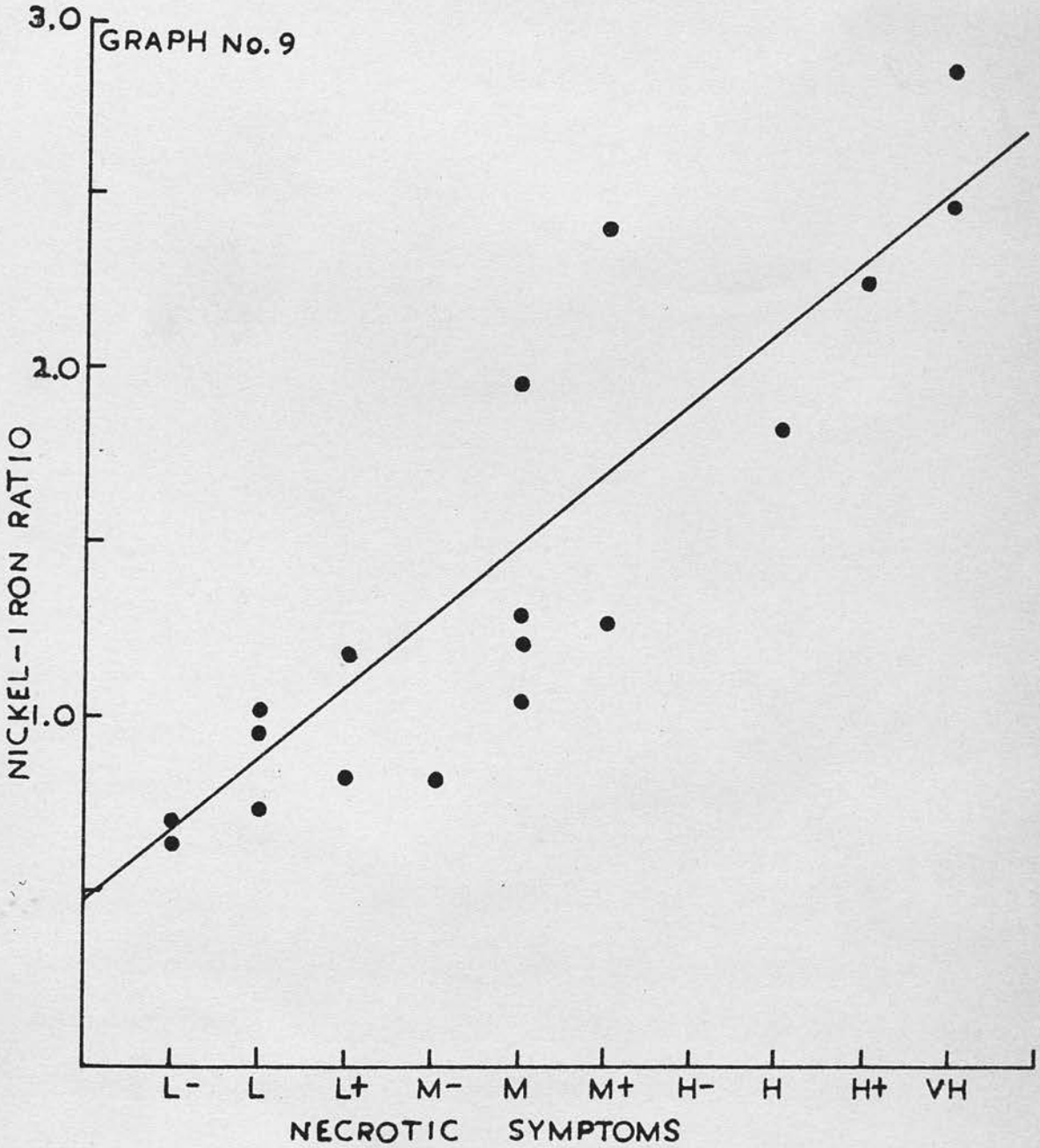
symptoms correlated with the nickel-iron ratio in the dry-matter. When this ratio was calculated from the results obtained in these three experiments, it was apparent that the two were connected linearly. The results have been regrouped in Table 34 below, in terms of increasing degree of symptoms and it is seen that in most cases, increase in symptom degree is associated with a higher nickel-iron ratio in the plant. The effect is shown graphically in graph No. 9.

TABLE 34

Relation between necrotic symptoms and Ni/Fe ratio

Experiment 1		Experiment 2		Experiment 3	
Symptoms	Ratio	Symptoms	Ratio	Symptoms	Ratio
0	0.50	0	0.49	L-	0.63
0	0.79	0	0.58	L	0.78
0	0.87	0	0.73	L	0.94
L-	0.70	L	0.85	L+	0.88
M	1.97	L+	0.78	M	1.05
M+	2.44	M-	0.82	M	1.22
H+	2.29	H	1.84	M	1.23
VH	2.89	VH	2.47	M+	1.31

Note: Ratios for healthy plants receiving no nickel in the nutrient solution (but found on analysis to contain from 4 - 6 ppm. nickel in the dry-matter) varied from 0.04 - 0.08.


GRAPH No. 9
3.0
2.0
1.0
NICKEL-IRON RATIO
L-
L
L+
M-
M
M+
H-
H
H+
VH
NECROTIC SYMPTOMS

DISTRIBUTION OF IRON IN NICKEL-TOXIC OAT PLANTS

Chemical analysis of nickel-toxic plant material had shown that the iron content was generally lower than for normal tissue, but gave no information on the distribution of the iron within the leaf. Autoradiography offers a convenient means of studying the distribution of various essential elements in the plant. One of the governing factors is the half-life of the isotope involved. For example, it would have been interesting to study the distribution of nickel in the plants, but the half-life of the only isotope (^{65}Ni) available at the time was only 2.5 hours which precluded its use in plant nutrition studies with oats. Iron, on the other hand, has isotopes of half-life 45 days and 2.94 years (^{59}Fe and ^{55}Fe respectively). Both isotopes occur in the radioactive iron solution supplied by A.E.R.E., Harwell but the low-energy particle emitted by ^{55}Fe probably takes no part in producing the autoradiographs, due to self absorption of the radiation in the plant tissue.

It was decided to grow oat plants in solution culture supplied with labelled iron at 1.2 ppm. (the 5 litres of nutrient solution in each crock would contain 1 microcurie of radioactive iron) either as ferric citrate or the Fe-EDTA complex. Autoradiographs of these plants would show whether either iron source had any influence on the distribution of iron within the leaf. Similar cultures, but supplied, in addition, with nickel at 1.2 ppm. would yield autoradiographs showing/

showing the effect of nickel on iron distribution. Periodic sampling was also used to see how distribution was affected by age of plant. With three crocks per treatment this allowed harvests (each of one crock) to be taken at the end of 14, 21 and 28 days. An additional crock per treatment was included using inactive iron to ensure that radiation damage did not influence (or invalidate) the experiment. These four crocks were harvested at the end of the 28-day period.

In the case of the ferric citrate treatments, the addition of the radioactive iron (in solution) presented no difficulty. It could be added to the ferric citrate stock solution without appreciably affecting the final concentration of iron in the nutrient solution. In the case of the iron complex it seemed desirable to add the radioactive iron to a solution of ferrous sulphate and then to complex with EDTA in the normal manner to ensure that the Fe-EDTA was uniformly labelled. Nutrient solutions were changed every seven days.

From the time of onset of toxic symptoms until about fourteen days after germination, young leaves unfolded chlorotic in all treatments receiving nickel. Thereafter, it was noticed that the young leaves emerged quite green. This had been noted before, although at a later stage of growth, both in the field and when carrying out the experiment on the rate of absorption of nickel with time, described in an earlier section.

Comparison/

Comparison of the yields of the inactive controls (see table below) and those of the third sampling, suggests that no radiation damage took place, although the reason for the greater reduction in yield due to nickel in the case of the former treatments is not clear. In the case of earlier samplings, the Fe-EDTA no-nickel pots outyielded those supplied with citrate, although the yield of 163.6 g. (third sampling) appears very low especially when considered along with the yield of 161.4 g. for the +Ni treatment and 218.7 g. for the inactive control. Incidently the symptoms seen in these inactive treatments did not resemble those normally associated with nickel-induced iron deficiency, and took the form of a diffuse pale yellowing of the leaves, accompanied by necrosis of a more severe character than that in corresponding radioactive cultures. No chlorosis was discernible in the young leaves because of the pale appearance of the whole plant. In addition these plants wilted badly on occasion and the slimy appearance of their roots suggested some form of fungal attack, which may have been responsible for the peculiar appearance of the tops.

In preparing the material for autoradiography, two or three plants from each pot were separated into leaves and stems and quickly dried on a hotplate between sheets of absorbent paper, placed between sheets of cardboard. The whole assembly was weighted evenly on top so that plants dried flat. The procedure followed that recommended by

Wittwer/

TABLE 35

Absorption of nickel and iron with time

	Yield per pot (g.)		Weight of dry roots (g.)	Concentration in dry matter (ppm.)		Total uptakes (mg.)		Toxic Symptoms	
	Fresh	Dry		Iron	Nickel	Iron	Nickel	Necrosis	Chlorosis
1st Sampling									
FeCi	31.84	2.80	lost	125	5	0.37	0.002	-	-
FeCi + Ni	29.95	2.67	in	75	61	0.22	0.18	L	M
Fe-EDTA	42.50	3.73	drying	97	4	0.39	0.002	-	-
Fe-EDTA + Ni	30.75	2.75		80	78	0.24	0.23	L	L
2nd Sampling									
FeCi	117.5	9.71	3.70	81	4	0.79	0.04	-	-
FeCi + Ni	103.5	8.45	3.25	79	96	0.67	0.81	M	0
Fe-EDTA	117.7	9.75	5.09	110	4	1.07	0.04	-	-
Fe-EDTA + Ni	87.5	7.52	2.75	81	78	0.61	0.59	M	0
3rd Sampling									
FeCi	163.6	17.41	3.28	92	1	1.60	0.02	-	-
FeCi + Ni	161.4	16.68	2.94	85	89	1.42	1.48	M	0
Fe-EDTA	221.3	22.31	5.16	87	4	1.94	0.08	-	-
Fe-EDTA + Ni	158.9	16.73	3.08	74	81	1.24	1.36	M	L
Inactive Controls									
FeCi	218.7	20.07	3.66	72	8	1.44	0.16	-	-
FeCi + Ni	108.2	10.44	1.68	54	97	0.56	1.01	H	M
Fe-EDTA	239.5	21.74	4.25	97	8	2.11	0.18	-	-
Fe-EDTA + Ni	124.2	11.73	1.78	70	94	0.82	1.11	M	M

Wittwer and Lundahl (146) which has subsequently been shown by comparison with other available methods, to be the most practical and to offer least chance of migration of nutrients during the drying process (Toth and Romney (128)). Ilford double coated X-ray film (industrial G) was used in making the autoradiographs. The leaf (or stem) was placed with the film between glass plates in a stout black paper envelope. The exposure time was approximately four weeks from an estimate previously made by means of a "count" of the fresh plant material using an end-window Geiger-Müller counter. The films were developed using I.D. 19 (5 minutes), washed (30 minutes), fixed (15 minutes), and finally washed for a further 30 minutes. The concentration of the radioactive isotope and hence of total iron was shown by the degree of blackening of the negative.

The autoradiographs so prepared were examined to see whether the different iron sources had produced a different pattern of distribution of the iron in the plant. Ferric citrate and Fe-EDTA were found to give similar autoradiographs, both of which showed that more iron was localised in the veins than in the interveinal tissue. There were indications that the Fe-EDTA plants contained more iron than those supplied with citrate but this was not borne out by analysis (see table above). When the autoradiographs of nickel-toxic material were examined it was again apparent that nickel had affected/

affected both sets of plants in a similar fashion irrespective of type of iron supply. In general, necrotic areas on the leaf could be matched precisely against areas in the autoradiograph low or lacking in iron. Similarly, chlorotic patches gave only a small blackening of the film, indicating the presence of much smaller amounts of iron than was found in normal tissue. The same pattern was noted in each autoradiograph irrespective of age of plant, although as was pointed out in an earlier section (see p. 72) some change in the nickel-iron relationship in oat plants takes place between thirty and forty days after germination and autoradiographs of plants of that age-group might have shown interesting differences.

It was thought that the effects of nickel on the distribution of iron could be shown up most readily by making photographic enlargements of each autoradiograph and the corresponding leaf. Four leaves (and their autoradiographs) from the final sampling were selected and treated as follows. A leaf and its autoradiograph were set up on the stage of an enlarger so that the two matched perfectly. A print was then made of each in turn by transmitted light on the same printing paper. This was necessary because of their different transmissions which called for different exposure times. In the enlargements shown here, the leaf is always on the right and the autoradiograph on the left of the picture. Necrotic areas on the leaf now appear black and are seen to correspond with dark areas in the autoradiograph i.e. to a low concentration of iron.

Photographic enlargements of Autoradiographs of oat leaves, showing the effect of nickel on the distribution of iron.

Healthy leaf

Necrotic leaf

1.2 ppm. iron as ferric citrate

0 - nickel

1.2 ppm. nickel

Photographic enlargements of Autoradiographs of oat leaves, showing the effect of nickel on the distribution of iron.

Healthy leaf

Necrotic leaf

1.2 ppm. iron as Fe-EDTA.

0 - nickel

1.2 ppm. nickel

As mentioned earlier, Vergnano considered that necrosis might be a very severe localised iron deficiency but had to modify this hypothesis on the results of certain experiments. These autoradiographs now seemed to suggest the same viz. that necrotic patches were very low or lacking in iron. It seemed possible that nutrients might well migrate out of such dying tissue, and indeed Millikan (91), has reported such a migration of manganese out of necrotic spots produced in cabbage by toxic levels of manganese. As the spots develop, they have a high manganese content but when necrosis becomes very severe and the tissues are dying, manganese moves out into the surrounding healthy tissue.

An obvious step was to analyse necrotic areas of oat plants and compare their nutrient content with that of the remainder of the leaf, and finally with that of healthy leaves. Oats growing on the basin soil at Whitecairns provided a plentiful supply of such material. Duplicate samples of fully expanded leaves were taken as follows: (a) healthy, (b) necrotic. Sample (b) was divided into three subsamples (i) necrotic patches (ii) remainder of these leaves and (iii) whole necrotic leaves. It proved impossible to exclude a certain proportion of green tissue in subsample (i) but the proportion was not high enough to influence the results if any real differences in nutrient content existed. The table below sets out the results obtained, as means of the two values obtained in each case.

T A B L E/

TABLE 36

Composition of oat leaves from Whitecairns

Material	% in dry matter				(ppm.) in dry matter	
	P	K	Ca	Mg	Fe	Ni
(a) Healthy leaves	0.23	1.81	0.62	0.34	114	36
(b) (i) Necrotic areas	0.23	2.23	0.45	0.27	84	45
(ii) Green areas	0.22	2.07	0.60	0.36	96	57
(iii) Whole necrotic leaves	0.24	1.83	0.63	0.38	99	57

Although the necrotic areas were not entirely devoid of iron (or other nutrients) as had perhaps been expected from an examination of the autoradiographs, there were some significant differences. The iron, calcium and magnesium contents of the necrotic tissue were lower while that of potassium was higher. Phosphorus, on the other hand, remained unchanged in concentration from healthy to toxic material.

FURTHER ASPECTS OF THE NICKEL-IRON RELATIONSHIP

The solution-culture experiments with oats described earlier in this section make it clear that the relative proportions of iron and nickel present in the nutrient solution determine the degree of toxicity, and therefore that the antagonism is mainly an external one, when referred to the plant. The possibility of an internal antagonism cannot, however, be ruled out.

In plant nutrition studies, particularly those dealing with phosphorus-iron relationships, certain investigators have adopted the procedure known as "split-root" culture which enables a particular element to enter the plant without interference due to precipitation or to antagonism by other elements. In this type of culture the root system is partitioned between separate containers which hold the nutrient solutions. The oat plant does not lend itself readily to this technique which calls for a fairly large root system. Tomato plants are well adapted for this purpose, however, on account of their extensive fibrous system arising from a diarch arrangement in the seedling stage (Haynes and Robbins (35)). Usually the root system is split some way up the stem to overcome possible 'wick' effects.

A small-scale split-root experiment using tomato was set up to obtain confirmation of the external nature of the nickel-iron antagonism.

The/

The nutrient solution used differed from the basic oat nutrient solution and had the following composition (per 10 litres): $NaNO_3$, 50 ml.; $Na_2HPO_4.12H_2O$, 150 ml.; K_2SO_4, 150 ml.; $Ca(NO_3)_2.4H_2O$, 30 ml.; $MgSO_4.7H_2O$, 15 ml.; Ferric citrate trace solution, 10 ml. Its pH was adjusted with acid to 5.0. The experimental design is sketched below.

	Low Iron	High Iron	
0-Ni	1.2 ppm. Fe	12 ppm. Fe	Normal culture
+Ni	1.2 ppm. Fe 2 ppm. Ni	12 ppm. Fe 2 ppm. Ni	Normal culture
+Ni	1.2 ppm. Fe | 2 ppm. Ni	12 ppm. Fe | 2 ppm. Ni	'Split-root' culture.

Tomato plants were raised from seed in sand-culture until they were about 5 - 6 in. high. Six uniform plants were selected, the roots were washed free from sand and the plants transferred to 5 litre crocks containing the appropriate solutions. The plants were supported on a plywood square (bitumen-painted) which had a central hole loosely plugged with non-absorbent cotton wool through which the plant roots were first passed. In the 'split-root' crocks the solution was held in polythene bags themselves placed in the crock. Each bag held 2.5 litres of the tomato nutrient solution (less iron), and nickel and iron were then added to bring their concentrations/

concentrations to the values shown in the diagram. The solution pH was checked daily and adjusted if necessary, to 5.0. Solutions were changed weekly.

Nicholas (97) has described the symptoms produced in tomato by excess nickel. The plants become very stunted, young leaves are chlorotic, and necrotic spots appear on old leaves. There may be death of the growing point in severe cases. The stems are also affected and in a later paper (99) the same author described how the plant may collapse due to extensive necrotic damage to the stem walls, and a bent habit results.

After six days, the young leaves of both normal cultures receiving nickel were chlorotic, the high iron treatment being less severely affected. Neither split-root plant showed any chlorosis. Four days later the first necrotic spots were seen in both +Ni (normal) cultures. At the end of two weeks, the 0-Ni cultures were growing well but high-iron had given a larger plant. In +Ni (normal) cultures the growing points were dying and the plants were very stunted. In contrast, the split-root cultures were growing vigorously although some apical chlorosis was apparent together with a slight mottle in the leaves of the low-iron plant. The reasons for this became apparent when the split-root systems were examined. In the 'nickel' side of the culture, roots were brown and failing to produce any secondary rootlets, whereas in the 'iron' side, roots were white and much longer with many secondaries.

It/

It therefore appeared as though nickel was acting as a root poison since in the +Ni (normal) cultures where the whole root systems were similarly affected, the plants were failing. Where only a part of the root system is in contact with nickel, the plant seems to be able to remain healthy by virtue of the nutrients absorbed through the remaining part of the system. In time the internal toxic effect of nickel (as evidenced by a leaf mottle) disappeared suggesting that the rate of absorption of nutrients (particularly iron) through the healthy roots was more than enough to offset any nickel absorbed through the other part.

The experiment was allowed to run for a further two weeks by which time the split-root cultures were as large and quite as healthy as the 0-Ni cultures(33 - 38 cm.high). The +Ni (normal)cultures were 18 - 20 cm. high. At harvest, tops and roots were separated and weighed fresh, and then again after drying (see Table 37). The analytical results are given in Table 38.

T A B L E/

TABLE 37

Yields from split-root experiment

Treatment	Tops (g.) Fresh	Tops (g.) Dry	Roots (g.) Fresh	Roots (g.) Dry
Controls				
Low iron	93.25	8.45	19.85	1.29
High iron	175.46	16.25	46.15	2.70
+ Ni				
Low iron	40.05	5.35	14.42	1.03
High iron	38.35	4.75	13.47	0.90
Split-root (+Ni)				
Low iron	141.24	13.22	(a) 34.93	1.64 *
			(b) 5.95	0.46
High iron	144.66	13.47	(a) 41.81	1.96
			(b) 6.10	0.45

* (a) no-nickel portion of root system,

(b) + nickel portion of root system.

TABLE 38

Composition of whole plants and roots from split-root experiment

Treatment	TOPS percentage in dry matter P	K	Ca	Mg	(ppm.) in dry matter Fe	Ni
Normal culture						
Low Fe	0.603	5.34	1.692	0.452	84	9
High Fe	0.606	4.93	1.633	0.334	75	12
Low Fe + Ni	0.615	3.96	0.945	0.253	51	82
High Fe + Ni	0.705	4.18	1.035	0.285	53	100
Split-root culture						
Low Fe + Ni	0.600	4.95	1.896	0.357	74	20
High Fe + Ni	0.562	5.01	1.771	0.304	85	19
	ROOTS				%	
Normal culture						
Low Fe	0.986	4.22	0.535	0.550	0.21	29
High Fe	2.558	3.75	0.463	0.385	0.31	42
Low Fe + Ni	0.861	4.34	0.480	0.214	0.43	758
High Fe + Ni	1.407	4.39	0.625	0.437	1.48	765
Split-root culture						
Low Fe + Ni (a)*	0.703	1.95	0.936	0.230	0.15	28
(b)	0.865	4.25	0.923	0.265	0.25	846
High Fe + Ni (a)	2.971	2.74	0.616	0.260	1.27	33
(b)	0.959	4.90	0.847	0.459	0.13	675

* (a) no-nickel portion of root system.

(b) +Ni portion of root system.

The major-element analyses for the tops of the plants from each treatment give the clearest indication of the extent to which the split-root cultures approached normal plants despite the fact that part of their root systems was supplied with nickel. The phosphorus, potassium and magnesium results, especially, reflect this similarity in composition. In the case of calcium, the uptake of which always appears to be peculiarly influenced by nickel, the levels found in the +Ni (normal) cultures are lower than for the no-nickel controls. This is the only case where such a reversal was noted, but it is no doubt connected with the severe injury induced in these plants by 2 ppm. nickel, which may have affected the absorption of calcium to a greater extent than that of the other nutrients.

The iron content of the tops of the split-root cultures is practically the same as for the no-nickel cultures, but the small effect on iron uptake produced by a tenfold increase in iron supply is most surprising. If we assume that the nickel found in the control plants is a measure of that present as impurity in the nutrient salts used, then the amounts of nickel translocated by the 'nickel' portion of the split-root cultures are seen to be very small indeed. Reference to the root analyses shows that these roots contained very high concentrations of nickel; higher, in fact, than those of the +Ni (normal) cultures which were found to have nearly ten times as much nickel in their tops. The amounts of other nutrients found in the tops of these normal cultures supplied with nickel suggest that the reason for the very small amounts of nickel being/

being translocated in the split-root cultures was not connected wuth the roots themselves but hinged on some other factor or factors within the plant.

The root analyses show that one of the results of a high iron supply is the large amount of phosphorus locked up in the roots. Potassium in the roots was consistently higher in the presence of nickel, and the analyses of the tops shows that there is more potassium in the controls, which possibly indicates an effect of nickel in reducing the movement of potassium out of the roots. The same effect was noted in the analytical results for oat plants in the nickel-iron ratio experiments, when the same comment was passed. The calcium and magnesium results show no consistent trends on this occasion. The control roots had relatively high amounts of nickel associated with them which seemed to increase with increased iron supply. When the solid ferric citrate used to prepare the trace solution was analysed it was found to contain 108 ppm. nickel*.

* I am indebted to Dr. D..J. Swaine for carrying out this determination.

EFFECT OF MOLYBDENUM ON NICKEL TOXICITY

In 1947, Millikan (88) reported that molybdenum supplied to plants suffering from metal-induced iron deficiency gave a beneficial effect and reduced the degree of chlorosis. Flax in solution culture was used as indicator plant and the metals tested included nickel, supplied in the nutrient solution at 0.1, 1, 2 and 5 ppm. Molybdenum was supplied as ammonium molybdate at levels of 0.1, 0.5, 1, 2, 5 and 10 ppm. In a later paper (91) the same author reported reduction in the manganese content of flax, peas, cabbage and tomatoes by 20 ppm. molybdenum in the nutrient solution.

Subsequently several workers have tried to reproduce this antidotal effect of molybdenum, but without success, although it is perhaps significant that all the later experiments differed from Millikan's original ones in that different indicator plants were employed or different levels were used in the nutrient solutions. Hewitt (42) in 1948, reported that molybdenum greatly accentuated the chlorosis produced in sugar beet by chromium, manganese, copper or zinc. In solution-culture experiments with soybean and flax, Warington (142) found that molybdenum intensified manganese-induced chlorosis.

Vergnano investigated the effect of adding molybdenum at levels of 0 - 25 ppm. to sand-cultured oats already receiving 2.5 ppm. nickel in the nutrient solution. Molybdenum was found to reduce the nickel content of the plant but not enough

to/

to affect the degree of toxicity symptoms which were in any case, increased at the highest rate of molybdenum supplied. More recently, Nicholas and Thomas (98) failed to obtain any improvement in cobalt-induced injury in soil-cultured tomatoes on adding molybdenum.

The number of negative findings so greatly outnumbered the original positive one, that it seemed unlikely that a further experiment would do more than add to this mass of negative data. The effect of excess molybdenum on the iron status of oat plants in solution culture appeared to warrant investigation so that any subsequent experiments to examine a possible nickel-molybdenum antagonism could be more readily interpreted.

In this first experiment the effect of 35 ppm. of molybdenum (as sodium molybdate) in the nutrient solution on the uptake of iron (from ferric citrate at 1.2 and 3.6 ppm. in the nutrient solution) was studied at pH 4 and 6. There were two crocks per treatment and solutions were changed every four days.

The plants were harvested at the end of 12 days but the level of molybdenum had been too low to produce severe toxic symptoms in the oats. Some reduction in growth had occurred but chlorosis was only evident in the young leaves of one treatment (low iron at pH 4). The table below gives the yields, and iron and molybdenum analyses.

TABLE 39

Effect of molybdenum on absorption of iron

Treatment	pH	Average height of plants (cm.)	Fresh yield per 2 pots (g.)	Concentration in dry matter (ppm.) Fe	Mo
Low Fe	4	30	39.5	60	12
Low Fe + Mo	4	22	15.2	27	645
High Fe	4	28	40.4	150	6
High Fe + Mo	4	18	12.9	84	798
Low Fe	6	30	47.7	70	5
Low Fe + Mo	6	30	43.0	86	471
High Fe	6	30	55.6	175	6
High Fe + Mo	6	30	49.5	86	400

One effect produced by molybdenum was an intensification in the colour of the plants, making them turn a dark blue-green, but there were no signs of the golden-yellow chlorosis typical of molybdenum toxicity. The reduction in growth and absorption of iron was more pronounced at pH 4 than at pH 6, which was in keeping with the higher absorption of molybdenum found at the lower pH value. This is the opposite to that seen in the field, where uptake of molybdenum by plants is greater at more alkaline pHs (Barshad (7)).

In/

In the second experiment, also in solution culture, all eight treatments (each of 2 crocks) were supplied with iron at 1.2 ppm. The effect of molybdenum (at 50 ppm.) on the toxicity produced in oats by 1.2 ppm. nickel was investigated at pH values of 4 and 6. The experiment ran for 18 days.

After four days plants receiving molybdenum were a dark blue-green, especially those at pH 4. No nickel-toxic symptoms appeared until the ninth day when signs of molybdenum toxicity, which affected the upper third of basal leaves turning them a golden-yellow were also evident. At pH 4, the necrosis due to nickel seemed less severe when molybdenum was also supplied in the nutrient solution. No similar beneficial effect was noted at pH 6.

Differences due to molybdenum were evident at the end of 15 days. At pH 4 plants were stunted and, where nickel was also present, were less necrotic than in the absence of molybdenum. At pH 6 however, molybdenum had not produced any stunting of the plants nor were the nickel-toxic symptoms improved by the addition of molybdenum. Plants at the lower pH, which were molybdenum-toxic, were found to have small pale golden-yellow roots. At pH 6, roots of similar treatments were yellowish in colour.

T A B L E/

TABLE 40

Effect of molybdenum on nickel toxicity symptoms

Treatment	pH	Fresh yield per 2 pots (g.)	Concentration in dry matter (ppm.) Fe	Ni	Mo	Toxic symptoms Necrosis	Chlorosis
Control	4	41.5	94	7	---*	0	0
+Ni	4	34.5	92	99	---	M	0
+Mo	4	11.0	78	9	965	0	M
+Ni +Mo	4	8.6	81	26	1076	0	0
Control	6	48.0	154	10	---	0	0
+Ni	6	29.8	76	118	---	M	H
+Mo	6	40.5	77	6	894	0	0
+Ni +Mo	6	28.8	62	100	1381	M	H

* not determined.

A striking fact which emerges from the above results is the relatively greater toxic effect of molybdenum at pH 4 than at pH 6, as judged by effect on yield, although the concentrations of molybdenum in the plants were not very different, and the uptake of molybdenum at pH 6 would be about three times that at pH 4.

The absorption of nickel has been much reduced by molybdenum at pH 4, although not at pH 6, and this presumably accounts for the lack of nickel toxicity symptoms observed at the lower pH. A smaller effect of nickel on the absorption of molybdenum at pH 6 was also noted. The results indicate the existence of a possible interaction between nickel and molybdenum under certain conditions, although in themselves unable to confirm or deny its existence.

CONCLUSIONS

The literature on metal-induced iron deficiency makes it clear that the resulting chlorosis stems from an upset in iron metabolism. The various reasons put forward to explain how heavy metals could interfere with normal iron metabolism have been outlined. Excess nickel produces necrosis in addition to chlorosis when supplied in excess to oat plants, and the experiments just described were carried out with a view to examining this antagonism in more detail. In particular, it appeared necessary to demonstrate the true site of the toxic action of nickel and to try and explain how a high iron supply prevents the appearance of toxicity symptoms.

The amount of nickel absorbed by oat plants increases with increasing pH in solution culture and the results in Table 20 (p. 88) show that this effect is still found with a high iron supply despite the fact that the plants so treated are not exhibiting any signs of toxicity. A further experiment investigated the effect of pH on the absorption of nickel in the absence of iron, the plants having been grown for a short initial period at optimum pH and iron supply. The type of iron was also varied being supplied either as ferric citrate or ferric-EDTA. Table 21 (p. 91) shows that in both cases, absorption of nickel increased with pH, although a slight reduction in absorption was noted at pH 7, in the case of the Fe-EDTA series.

There/

There were by now indications that the relative amounts of nickel and iron in the nutrient solution determined the degree of toxicity and this contention was confirmed in an experiment in which plants were raised on various levels of iron before being transferred to solutions containing nickel. The same degree of symptoms were found irrespective of initial treatment, hinting tat the external nature of the nickel-iron antagonism. A further "transfer" experiment gave additional positive proof of this. In this case the plants were cultured during the initial period on an optimum iron supply before being transferred to solutions containing nickel and various iron levels. The highest absorption of nickel and degree of toxicity occurred where nickel and no iron was supplied, while no symptoms appeared where nickel and a high level of iron were present in the nutrient solution.

The relationship between the nickel-iron ratio in the substrate and the toxic symptoms produced in oat plants grown in these solutions was next examined. It was found that for cultures having the same nickel-iron ratio, higher symptoms could be expected in plants supplied with the higher amount of nickel. This series of experiments yielded data which showed that a positive correlation existed between degree of toxic symptoms and the nickel-iron ratio found in the plant. Root analysis showed that the high concentrations of iron found in nickel-toxic roots were not markedly higher than those associated with healthy roots. The concentration of iron in the/

the tops of healthy or nickel-toxic plants supplied with a wide range of iron levels in the nutrient solutions showed remarkably little variation.

The major-element composition of roots and tops of plants from these experiments showed interesting differences due to nickel. In the case of the tops, the effect of nickel in greatly increasing the concentration of calcium was noteworthy, although difficult to explain without further detailed experiments. A similar effect was noted in the case of magnesium. The potassium content of the roots was much higher in the +Ni plants, although no wide variation in the level in the tops had been noted. When these results are recalculated in terms of total potassium content it is found, on the average, that while the content of the +Ni roots was about 10% higher than that for normal roots, the potassium content of the tops of +Ni plants was only about two-thirds that of healthy plants, suggesting that nickel interferes in the translocation of potassium.

The distribution of iron in the leaves of nickel-toxic oat plants was studied by means of autoradiography. In control plants, ferric citrate and Fe-EDTA gave the same iron distribution pattern in the leaf, there being more iron in the veins than in the interveinal tissues. In the presence of nickel, iron from either source was found to be reduced in chlorotic tissue and to be present in very low concentration in necrotic tissue. When necrotic areas from oat plants

grown/

grown on Whitecairns basin soil were analysed for iron, nickel and major nutrients it was found that while iron was somewhat lower than in the green parts of the leaf, other nutrients did not vary to any great extent.

Final confirmation of the external nature of the nickel-iron antagonism was obtained in a 'split-root' experiment with tomato, which is more suitable than the oat for this type of culture. The level of nickel supplied (2 ppm.) proved to be very toxic and speedily poisoned the roots of the normal cultures, and those in the +Ni portion of the split-root cultures. In the case of the normal culture receiving nickel, since the whole root system was affected, the plants made little further growth and high concentrations of nickel were found in the tops, although their major-element content was not unduly affected. In contrast to these results, and in spite of the fact that the +Ni portion of the root system of the split-root cultures were found to contain just as much nickel as the +Ni normal cultures, the nickel contents of the tops of the former were very low, in fact only about twice that of the controls. Even when differences in yields of dry matter are taken into account, the nickel uptake by the split-root cultures is only about half that of the +Ni normal cultures (0.26 and 0.26 mg. as compared with 0.44 and 0.48 mg.).

It is becoming a generally accepted fact that iron and other trace elements probably do not move in the plant as simple ions. Their efficient translocation appears to depend on chelation or complexing by organic acids present in the plant.

The/

The results obtained in iron nutrition studies with aminopolycarboxylic acids (EDTA being that most frequently used) have added fresh evidence on this score (143). Nickel may also be dependent on a similar type of mechanism for transport within the plant and may well compete with iron, resulting in lowered translocation of iron to young tissues and so to the production of chlorosis. Even so, it is not clear why the translocation of nickel from roots having approximately the same nickel content should be so markedly different in the case of the tomatoes mentioned above. Complete inactivation of the root system by nickel cannot have been involved since those in the +Ni normal cultures absorbed and translocated major nutrients in amounts little different from those found in control plants. It must be concluded that the good growth of the no-nickel portionsof roots in the split-root cultures resulted in its being preferentially used by the plants as the main channel for nutrient absorption.

The results of the limited experiments carried out to study a possible nickel-molybdenum interaction, disagree within themselves. A beneficial effect was found at pH 4 but not at pH 6, emphasising that it was not surprising that different investigators, using different techniques, were unable to obtain satisfactory agreement regarding the existence of the antagonism. Further detailed work would be required before a definite answer could be obtained.

THE RELATIONSHIP BETWEEN NICKEL TOXICITY AND MAJOR NUTRIENT SUPPLY

The effect of increase in soil pH on nickel-toxic symptoms and the uptake of nickel has been discussed in a previous section. In a series of field experiments at Whitecairns, Hunter and Vergnano (56) showed that the most satisfactory corrective treatment for these nickel-toxic soils is the application of lime, the resulting reduction in toxicity symptoms being due to reduced availability of soil nickel. In the course of further investigations using soil- and sand-cultures, certain discrepancies were observed in the results obtained. This suggested the existence of a relationship between the effect of nickel and the supply of major nutrients, a possibility strengthened by the abundant evidence of a similar relationship existing between major elements (especially calcium) and certain other trace elements (for example, manganese and aluminium, (Hewitt (39, 40)).

Subsequent experiments carried out by Vergnano (133) indicated that a high level supply of calcium to oats in sand culture reduced the necrotic symptoms due to nickel, a result comparable with that seen in the field. On the other hand an increase in the amount of phosphorus supplied caused an increase in the degree of symptoms. The experiments to be described now were carried out to establish the relationship between level of supply of major nutrients (nitrogen, phosphorus/

phosphorus, potassium, calcium and magnesium) and nickel toxicity in sand-cultured oat plants. Two types of experiment were used. In the first, nutrients were supplied at low and normal levels, and in the second at normal and high levels. The normal levels used were those of the basic oat nutrient solution which was known to contain nutrients in amounts adequate for healthy growth.

Nutrients at low levels

An experiment of this type was carried out by Vergnano (132) in which nickel at 2.5 ppm. or 5 ppm. was supplied to oats grown in a series of nutrient solutions whose effects on nutrient status had been ascertained beforehand. Five solutions were used; in each, four of the major nutrients were supplied in normal amount while the fifth was supplied at a level which gave a low concentration of that nutrient in the plant without actually producing deficiency symptoms. The experiment ran for 53 days before harvesting and when the plants were analysed for nickel it was found, particularly where 5 ppm. had been supplied, that the expected differences in uptake produced as a result of varying the major nutrients supply were not large. The reason for this appeared to be due to the fact that this high-level supply of nickel over such a lengthy period had overshadowed the presumably smaller effects of major elements on its uptake. With 2.5 ppm. nickel, however, certain effects were noted. The plants least affected by nickel toxicity were those in the phosphorus-low treatment/

treatment while those grown on calcium-low or magnesium-low nutrient solutions were most severely affected. Potassium-low and nitrogen-low plants were more severely affected than control plants receiving the same level of nickel but supplied with the basic oat nutrient solution. It was possible then to day that the trends found here operated in the same direction as those noted in the field, where increase in calcium had reduced symptoms and increase in phosphorus had increased symptoms.

There seemed two possible ways of obtaining satisfactory results from an experiment of the type described above. These were (a) grow the plants for a much shorter period, say 14 - 20 days or (b) raise the plants on the differential major-nutrient solutions for, say, 40 days and then supply nickel for 7 - 10 days at the same levels as used previously. Thus nickel would be supplied to plants of the required nutrient status and any resultant differences in nickel uptake should thereby still be apparent at harvest. It was realised that nickel absorption by these relatively mature plants would probably be small but it was hoped that if each major nutrient affected this differently then the effect due to individual nutrients would be shown up.

It was decided to follow the second alternative and grow oat plants in sand culture for 50 days, nickel at 2.5 or 5 ppm. being supplied for the last 10 days only. The nutrient solutions used were similar to those used by Vergnano for this type of experiment and their composition is given in the accompanying table.

TABLE 41

Composition of nutrient solutions.

Stock Solution	ml. stock solution per 10 l. nutrient solution					
	Normal	N-low	P-low	K-low	Ca-low	Mg-low
$NaNO_3$	15	--	15	15	15	15
$Na_2HPO_4 \cdot 12H_2O$	20	20	5	20	20	20
K_2SO_4	20	20	20	3	20	20
$Ca(NO_3)_2 \cdot 4H_2O$	15	15	15	15	--	15
$CaCl_2$	2.5	2.5	2.5	2.5	--	2.5
$MgSO_4 \cdot 7H_2O$	15	15	15	15	15	--
Ferric citrate	10	10	10	10	10	10

The sand used in this experiment was leached overnight with hot 15% hydrochloric acid to remove major nutrients, particularly calcium. Three pots per treatment were used. As growth proceeded it was apparent that while the nitrogen-low and phosphorus-low treatments were giving smaller plants than normal (potassium-low plants were similar in size to the normal ones), plants in the calcium-low and magnesium-low treatments were bigger than those grown on the normal nutrient solution. Vergnano also mentions this effect but is unable to explain it, although her analyses and those reported here did actually show low concentrations of calcium and magnesium in these plants, which was what was intended.

At the end of 40 days, the six pots in each treatment were grouped in three pairs, which continued to receive the same nutrient solution but now containing 0, 2.5 or 5 ppm. nickel/

nickel. A week later, certain 5 ppm. nickel treatments were showing symptoms of nickel toxicity. These were nitrogen-low and calcium-low (both necrotic) and potassium-low (chlorotic). At harvest, three days later, none of the plants which had had 2.5 ppm. nickel showed any toxic symptoms although all pots in the calcium-low group had mildly chlorotic young leaves which was presumably due to a calcium deficiency. The total production of dry-matter per treatment was assessed, and major elements, nickel and iron determined in the dry-matter of whole plants. The results in the table below are set out in groups of three. The first set of figures in each group refer to the control, the second and third to plants receiving 2.5 and 5 ppm. nickel respectively.

It was obvious at the time of starting the nickel treatments that the variation within treatments was too great for the final results to yield any definite information on the major-nutrient effects. The table below confirms this observation. The results will therefore not be discussed at great length, since the more positive findings of the factorial experiment described next permit of a more rigorous treatment. Again, the levels of nickel found in the control plants of this experiment were amongst the highest found in either sand or solution-culture work carried out in the course of this investigation. They arose, presumably, as the result of the presence of higher amounts of nickel than usual in the nutrient salts used to prepare the nutrient solutions./

TABLE 42

Results of low major nutrients experiment

Treatment	Average yield dry matter per pot (g.)	Concentration in dry matter (%) N	P	K	Ca	Mg	(ppm.) Fe	Ni	Toxic symptoms Necrosis	Chlorosis
Normal	36.5	2.13	0.198	2.60	0.187	0.220	35	17	-	-
	36.4	2.22	0.195	2.44	0.198	0.225	33	41	0	0
	30.9	1.85	0.184	2.24	0.173	0.215	23	64	0	0
N-low	24.4	1.77	0.259	3.13	0.227	0.273	24	17	-	-
	26.1	1.77	0.228	2.88	0.175	0.233	21	56	0	0
	28.2	2.02	0.234	3.00	0.540	0.260	29	103	M	0
P-low	19.5	1.82	0.091	2.88	0.187	0.155	22	17	-	-
	14.3	1.80	0.096	2.88	0.156	0.153	26	41	0	0
	18.0	1.68	0.096	2.65	0.163	0.150	13	60	0	0
K-low	40.4	1.90	0.179	1.04	0.390	0.238	32	14	-	-
	38.4	1.89	0.171	0.84	0.450	0.248	27	38	0	0
	33.4	2.15	0.176	0.76	0.198	0.200	24	54	0	M
Ca-low	39.9	2.05	0.160	2.00	0.102	0.208	23	11	-	M
	37.3	2.09	0.165	1.92	0.098	0.348	24	39	0	M
	35.2	2.07	0.157	1.88	0.090	0.235	23	95	L	M
Mg-low	32.0	1.86	0.182	2.44	0.430	0.050	32	14	-	-
	34.4	1.86	0.171	2.36	0.220	0.043	24	40	0	0
	38.5	1.64	0.157	2.08	0.400	0.040	28	55	0	0

solutions. It was subsequently shown that the stock solutions could be freed of such nickel impurities by the use of dithizone in carbon tetrachloride at alkaline pH, following the procedure outlined by Hewitt (45).

Although the nickel levels are higher than expected, it is improbable that the experiment is invalidated for this reason. The nickel uptake figures show that the nitrogen-low and calcium-low absorbed considerably more nickel than did the control, phosphorus-low, potassium-low or magnesium-low plants. The major-element analyses show that the individual nutrient solutions used had the desired effect in lowering the concentration of the major nutrient concerned in the plant.

Nutrients at high level

A factorial design (see Table 3 of the Appendix) was used involving two levels (normal and high) of each of the five major nutrients (nitrogen, phosphorus, potassium, calcium and magnesium) and two levels of nickel (0, and 2.5 ppm.). All combinations of these six, two-level factors gave 64 treatments and each was applied to a unit of three pots. A smaller block size was desirable in order to give a more accurate estimate of experimental error. The 64 treatment units were divided into 4 blocks each of 16 treatments. In order to achieve this three of the third-order interactions, (NPKNi, NCaMgNi and PKCaMg), which in any case were likely

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to be small, were identified with differences between blocks. The usual randomisation procedure was carried out in allocating groups of treatments to the blocks, and treatments to the units within a block. Since only one replication was used an estimate of experimental error with 19 degrees of freedom was obtained from the remaining third-order and higher-order interactions.

The composition per litre of the major-element stock solutions, and volumes required to prepare 10 litres of each nutrient solution are given in Table 43 below. The table is given as concisely as possible, but it is understood that 32 solutions were involved, produced by varying each of the major nutrients one at a time. E.g. in the treatment $N_2P_1K_2Ca_1Mg_1$ the amounts of stock solution for P, Ca and Mg would be found under "normal" and those for N and K under "high". "Normal" and "high" correspond to "level 1" and "level 2" respectively which are used in reporting results.

Ferric citrate (at 1.2 ppm. in the nutrient solution) was used as iron source. The pH of each solution was adjusted by sulphuric acid to 5.5. The experiment was carried out in the cage and the plants were grown for 34 days before harvesting. The full analytical data will be found in Appendix I (Table 4). In order to present the results in as concise a form as possible, tables have been drawn up which are derived from a statistical analysis of the full analytical data. Their layout is explained under the relevant heading.

Yield/

TABLE 43

Composition of nutrient solutions

Salt	g./l.	"NORMAL" ml./10 l.	"NORMAL" ppm. of element	"HIGH" ml./10 l.	"HIGH" ppm. of element
$NaNO_3$	250	15	138*	75	310*
$Na_2HPO_4.12H_2O$	50	20	8.7	140	61
K_2SO_4	90	20	81	120	486
$Ca(NO_3)_2.4H_2O$	430	15	151**	15	602**
$CaCl_2$	453	2.5	--	30	--
$MgSO_4.7H_2O$	250	15	37	75	185

* including N supplied by $Ca(NO_3)_2.4H_2O$.

** including Ca supplied by $CaCl_2$.

Yield

In the yield tables given below, the left-hand table shows the effect of change in level of supply of major nutrients on yield. The entries are thus means of 32 observations e.g. 83.3 g. is the mean yield of the 32 treatments receiving nitrogen at the lower level. Similarly the yield of 100.9 g. is the mean derived from the 32 N_2 treatments. Again the effect of nickel, averaged over all treatments has been to reduce yield by 11.9 g. (98.1 - 86.2 g.). The right-hand table gives the reduction in yield due to nickel at the two levels of each of the major nutrients*. From this the effect/

* The figures are therefore differences between means of 16 treatment units in each case i.e. at level N_1 ,- 13.0 g. is the difference between the 32 N_1 treatments, 16 having nickel, and 16 without nickel.

effect of increasing each major nutrient in turn on the yield reduction can be seen. Thus increase in nitrogen has no great effect (yield reduction is increased from -13.0 to -10.8 which is not significantly different from the average effect of -11.9 g.). Increase in calcium, on the other hand has a very beneficial effect, offsetting the nickel reduction from -15.9 g. at Ca_1 to -7.9 g. at Ca_2.

TABLE 44

Yield of dry matter (g.) per treatment

Factor	Mean yield (g.) level of factor 1	2	Factor	Nickel effect (-11.9 g.) level of factor 1	2
N	83.3	100.9***	N	-13.0	-10.8
P	86.8	97.5***	P	- 8.8	-15.0*
K	90.2	94.1**	K	-14.7	- 9.1*
Ca	93.0	91.3	Ca	-15.9	- 7.9**
Mg	93.8	90.4*	Mg	-13.9	- 9.9
Ni	98.1	86.2***			

SE of difference = ± 1.2 SE of difference = ± 2.4

*** Significant at 0.1% level.

** Significant at 1% level.

* Significant at 5% level.

When variation in yield due to differences in major-element supply was examined it was found that nitrogen, phosphorus and potassium increased yield while magnesium reduced it. With the addition of nickel a marked reduction in yield resulted./

resulted. Increase in calcium and potassium offset this reduction in yield due to nickel but increase in phosphorus supply produced a further decrease.

Nickel toxic symptoms

Signs of nickel toxicity first appeared two weeks after the start of the experiment. It was noticed that symptoms in high phosphorus treatments were generally more severe than in corresponding low phosphorus treatments, and that high nitrogen and calcium had each reduced the degree of necrosis. Marks were allotted on the scale of 0 (no symptoms) to 10 (very severe necrosis). When the experiment was harvested only treatment $N_1P_2K_1Ca_1Mg_1$ rated 10 while certain treatments involving high nitrogen and calcium had scores of only 2 or 3 (see Table 4 of the Appendix). The control treatment with major nutrients supplied at normal level scored 9. In the absence of applied nickel no symptoms were observed. The data thus consisted of observations on the 32 treatments which had received nickel. Analysis of variance was carried out on these visual scores and an estimate of error variance with 13 degrees of freedom was derived from high-order interactions. The results in the table below, show the effects of the five major nutrients on necrotic symptoms. The figures given in the table are the mean of 16 observations at each level of the factors.

T A B L E/

TABLE 45

Nickel toxic symptoms (necrosis).

Factor	mean score level of factor 1	2
N	4.6	1.6***
P	2.6	3.6**
K	3.7	2.5**
Ca	4.1	2.1***
Mg	4.2	2.0***

SE of difference = $\pm$ 0.34

An increase in nitrogen, calcium or magnesium supply has given large reductions in necrotic symptoms. Increase in potassium supply also significantly reduced the necrotic symptoms. In contrast to these results, phosphorus has had the opposite effect and increased the symptoms.

Chlorotic symptoms were also recorded and marks were allotted on a scale 0 (no symptoms) to 5 (severe chlorosis). Data were obtained only for these treatments which received nickel. Mean values for the two levels of each factor are given in Table 46.

Phosphorus was found to increase chlorosis but increase in the other major-element cations did not significantly affect the level of chlorosis. Nitrogen, however, was found to reduce chlorosis but this result should be treated with some reserve, in view of the possibility that chlorosis was masked in high-nitrogen plants. In the analyses of the two sets of scores the error variances appeared homogeneous.

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TABLE 46

Nickel toxic symptoms (chlorosis).

Factor	mean score level of factor 1	2
N	3.1	2.1***
P	1.8	3.4***
K	2.6	2.5
Ca	2.7	2.4
Mg	2.8	2.4

SE of difference = ± 0.25

In the case of necrotic scores a more rigorous analysis was carried out. The results of this analysis confirmed those already given.

Analytical results

The plant analyses exhibited features common to most nutrition experiments; a higher level of an element in the nutrient solution resulted in an increased concentration of that element in the plant, and sometimes caused a reduction in the uptake of other ions. No useful purpose is served by reporting these major-element interactions in full since they are merely incidental to the main investigation. Table 47 below contains the relevant analytical data, expressed in terms of total uptake of nutrients. These figures are derived from the values in Table 4 of the Appendix by taking yield differences into account (total uptake/

uptake (g. or mg.) = concentration in dry matter (per cent or ppm.) x yield of dry matter (g.)). The upper part of the table sets out the effect of nickel on the mean uptakes of each of the major elements and iron.

The data in the lower part of the table shows the effect of variation in major nutrient supply on this nickel effect. The layout can probably best be explained by discussing one column in detail. Thus nickel markedly reduces the nitrogen content of the plants (3.72% in the absence of nickel compared with 3.22% where nickel is supplied). This much is evident from the top-half of the table. Turning now to the lower half we see that the only significant change produced by variation in rate of supply of the other major nutrients is brought about by increasing potassium. The overall nickel effect is to reduce nitrogen content by 0.5 g. When this 0.5 g. reduction due to nickel is broken down in terms of variation in potassium supply, increase in potassium is found to offset it (-0.67 g. at K_1 and -0.33 g. at K_2). Increase in other nutrients (with the exception of phosphorus) also produce beneficial effects although none is significant. Increase in phosphorus on the other hand, further reduces the nitrogen content.

The uptake of iron and the major elements (with the exception of calcium) have been markedly reduced by nickel. Where nickel reduced the uptake of an element the effect was generally greater when the element was supplied at the higher level/

TABLE 47

The effect of Nickel on the Mean Uptake of Major Nutrients

Factor \ level of factor	Nitrogen 1	Nitrogen 2	Phosphorus 1	Phosphorus 2	Potassium 1	Potassium 2	Calcium 1	Calcium 2	Magnesium 1	Magnesium 2	Iron (mg.) 1	Iron (mg.) 2	Nickel (mg.) 1	Nickel (mg.) 2
Ni	3.72	3.22***	0.629	0.534***	4.31	3.65***	0.280	0.317***	0.238	0.220***	2.33	1.67***	--	--
SE of difference	±0.052		±0.0094		±0.053		±0.0040		±0.0043		±0.089		--	
Nickel effect	-0.50 g.		-0.095 g.		-0.66 g.		0.037 g.		-0.018 g.		-0.66 mg.		--	
N	-0.58	-0.42	-0.111	-0.079	-0.76	-0.56	0.039	0.035	-0.016	-0.020	-0.65	-0.67	6.55	6.56
P	-0.44	-0.56	-0.041	-0.149***	-0.60	-0.72	0.036	0.038	-0.015	-0.021	-0.53	-0.79	6.21	6.90***
K	-0.67	-0.33*	-0.123	-0.067***	-0.53	-0.79*	0.037	0.037	-0.025	-0.011	-0.74	-0.58	6.51	6.60
Ca	-0.60	-0.40	-0.109	-0.081	-0.78	-0.54*	0.016	0.058***	-0.017	-0.019	-0.81	-0.51	6.36	6.75
Mg	-0.54	-0.46	-0.094	-0.096	-0.82	-0.50***	0.060	0.014***	-0.031	-0.005***	-0.86	-0.46*	6.70	6.41
SE of difference	±0.105		±0.0188		±0.105		±0.0079		±0.0087		±0.179		±0.224	

level (phosphorus and potassium) although with magnesium the effect was greater at the lower level of magnesium supply. The increase in uptake of calcium due to nickel was greater where high calcium was supplied.

Turning to other first-order interactions, reductions in nitrogen and phosphorus uptake were offset by a high level supply of potassium, while reduction in potassium uptake produced by nickel was less where calcium or magnesium supply was high. A similar effect was produced by high level magnesium on the reduced iron uptake.

Values for nickel uptake at the two levels of each of the major nutrients were obtained from the 32 treatments where nickel was supplied. Where no nickel was supplied that found in the plants was derived from the nickel present as an impurity in the nutrient salts used. The average uptake for the 32 (no-nickel) treatments was 0.70 mg. in contrast to an average uptake of 6.55 mg. by plants supplied with 2.5 ppm. nickel in the nutrient solution. Variation in the level of supply of the major nutrients, except phosphorus, had no great effect on the uptake of nickel. Phosphorus however, significantly increased the uptake of nickel, a finding in agreement with the increased severity of symptoms resulting on an increase in the level of supply of substrate phosphorus.

The/

The results in the foregoing tables show that nickel very significantly depressed yield of dry matter and the uptake of the major nutrients with the exception of calcium where uptake was increased. Vergnano (132) using fresh-tissue extracts of oat stems, found that nickel reduced nitrate content and caused accumulation of phosphate.

The increased uptake of calcium by plants receiving nickel had been noted before when fresh-tissue extracts were being used as a measure of nutrient status. At the time no particular importance was attached to this result because of the possibility that rate of extraction of calcium from nickel-toxic tissue differed from that for normal tissue. Ash analysis has given the same results, which means that a toxic trace-element is affecting the uptake of a major element. While it is difficult to advance a possible explanation for this phenomenon, its results are in keeping with the observed effect of calcium on toxicity symptoms. Calcium (of the cations) is found to be the most active in reducing the toxic symptoms produced by nickel and this suggests that the two effects are related. It is interesting to note that the symptom reductions brought about by increased magnesium or potassium supply are not accompanied by increased uptakes of these elements. While in considering factors leading to reduction in nickel toxicity symptoms, the ability to reduce the uptake of nickel is of prime importance, their effect on the absorption of iron must also be considered,

since/

since this will affect the nickel-iron ratio in the plant and therefore the degree of symptoms produced. Thus while increase in potassium and calcium both cause increases in nickel uptake, magnesium reduces uptake of nickel. Again, although all three elements exert a beneficial influence on iron uptake by offsetting the reduction in uptake due to nickel, only magnesium does so to a significant extent thereby making the largest contribution toward affecting the nickel-iron ratio in the plant.

The reduction in uptake of other elements is more in keeping with the observed toxic action of nickel on roots, which become stunted and brown in colour with supression of secondary root development. As was pointed out in the discussion of the solution-culture experiments, where various substrate nickel-iron ratios were employed, only a small and constant amount of the iron absorbed is translocated.

The effect of the various treatments in reducing or increasing toxicity symptoms is not reflected in the uptake of nickel. When treatment differences are eliminated no correlation is found between symptoms and nickel level in the plant. Recourse was then made to the nickel-iron ratio which had earlier been shown to correlate well with degree of toxic symptoms. On this occasion, when applied to these results, only a small correlation was found due to the fact that treatment is affecting symptoms and the ratio in a like manner. When the effect of treatment is removed statistically/

statistically the correlation between symptoms and the nickel-iron ratio is not significant. This does not mean that the ratio approach is necessarily incorrect but rather that this experiment is not capable of - and indeed was not designed with a view to - investigating the relationship between the two. In the case of symptoms of more than moderate severity, a graph of symptoms versus nickel-iron ratio suggests that a definite relationship exists. For mild symptoms, the wide range of ratios which have been produced as the result of treatment differences made demonstration of the correlation difficult.

These experiments*, while confirming earlier sand-culture results agree also with the results observed in the field following the application of fertilizers to the Whitecairns nickel-toxic soils. This raises the question of applicability of these sand-cultures results to explain field observations. If we consider the two main effects viz. the beneficial effect of increased calcium supply and the adverse effects of a high phosphorus supply, it seems reasonable to assume that the latter effect, since it probably occurs within the plant, operates in the same way under both sets of conditions.

* The results of the two experiments just discussed, have been submitted as a joint paper with R.H.E.Inkson (Statistician, Macaulay Institute for Soil Research) and have been accepted for publication (24).

The application of calcium to a soil produces other effects, notably change in soil pH, besides increasing the level of calcium in the soil. Hunter and Vergnano (56) have shown that calcium sulphate has not the beneficial effect of calcium carbonate in reducing toxicity symptoms in plants growing on nickel-toxic soils, which suggested that the effect was due to a reduction in available nickel following change in soil pH. In sand-culture, however, the position is quite different since no pH change is involved following the increased supply of calcium in the nutrient solution, and furthermore the "available" nickel level in the nutrient solution is unaffected by changes in its cation content. One reason for the reduction in toxic symptoms could be a reduction in the uptake of nickel because of the increased cation content of the nutrient solution. The divalent cations Ca^{++} and Mg^{++}, would be expected to exert a relatively greater beneficial effect than K^{+} and this is found here. The uptake of nickel, however, is not significantly reduced by increases in any of these cations although this does not rule out the possibility that the rate of absorption of nickel was not reduced under these conditions.

The phosphorus-nickel relationship can possibly best be explained in terms of their separate effects on iron metabolism. It has already been shown that a high-level supply of iron will prevent the appearance of toxicity symptoms/

symptoms in oat plants. Increase in phosphorus supply in the presence of a fixed iron supply would be expected to reduce the absorption and translocation of iron, and this in turn would increase the nickel-iron ratio in the plant and so produce an increase in toxicity symptoms. This is the simplest explanation and further experiments would be required to test this hypothesis.

The interaction between phosphorus and another heavy metal, cobalt, has been the subject of a recent paper by Nicholas and Thomas (98). Using tomato grown in soil they found that the toxic symptoms produced by the addition of cobalt were alleviated on increasing the phosphorus supply. The paper records no data on the effect of change in phosphorus level on the availability and uptake of the added cobalt following the additions of fertilizer phosphorus. A priori, the results suggest that an increase in phosphorus supply decreased the level of cobalt available to the plants. In contrast to this, Askew and Dixon (6) have found that uptake of cobalt was increased following the addition of phosphorus as superphosphate. Their experiments, carried out in soil culture, investigated the effect of lime and superphosphate additions on the uptake of added cobalt by various pasture plants. Lime was found to depress uptake of cobalt.

Hunter and Vergnano (57) have examined the effects of various heavy metals on oat plants and concluded that nickel and/

and cobalt behave very similarly in their toxic action, the main difference being one of degree - cobalt being less active than nickel. In view of the above findings it was thought worth while to repeat the relevant treatments in the main factorial experiment involving low and high phosphorus, but to supply cobalt in the nutrient solution at 5 and 20 ppm. to oats in sand culture.

TABLE 48

Effect of high phosphorus supply on cobalt toxicity

Treatment	Yield of dry matter (g.)	(%) P	ppm. Co	ppm. Fe	Total uptakes P (g.)	Total uptakes Co (mg)	Total uptakes Fe (mg)	Symptoms Necr.	Symptoms CHh.
Normal P									
Control	10.65	0.66	*	65	0.07	----	0.69	0	0
+5 ppm. Co	9.18	0.72	162	59	0.07	1.48	0.54	0	M
+20 ppm. Co	5.38	1.25	1075	45	0.07	5.78	0.24	M	H
High P									
Control	13.41	1.29	--	63	0.17	----	0.84	0	0
+5 ppm. Co	7.94	2.57	176	54	0.20	1.39	0.42	0	M+
+20 ppm. Co	5.13	2.49	955	41	0.13	4.90	0.21	M	H

* not determined.

The experiment was carried out in the greenhouse using artificial illumination. 5 ppm. cobalt produced a prominent interveinal chlorosis in the oat plants at both rates of phosphorus supply. Plants receiving 20 ppm. cobalt had necrotic leaf margins and tips in addition to being severely chlorotic./

chlorotic. The appearance of the plants changed also; leaves were narrow and the habit became spiky. The increase in symptoms resulting from an increase in phosphorus supply was not so definite as was obtained with nickel. Chlorotic symptoms at 5 ppm. cobalt level definitely increased in severity on increasing the phosphorus supply, but at the higher cobalt level no change in symptoms was seen. From Table 48 it will be seen that cobalt (at both levels) produced a greater reduction in yield of dry matter (per treatment of 3 pots) when high phosphorus was supplied. Uptake of cobalt was not markedly affected by rate of phosphorus supply, while iron uptake was reduced with increasing cobalt in the substrate.

While certain of the results of this experiment are not so clear-cut as those found for nickel, there are features common to both sets of data which support the conclusions drawn about the similarity between nickel and cobalt toxicity. The results of Askew and Dixon, in so far as they are directly comparable, are in agreement with those reported here in that they show that the interaction between the metal and phosphorus is operating in the same direction viz. in increasing the uptake of the metal and in aggravating the toxic symptoms produced. Minor variations in degree, but not in kind, are to be expected when different plant species and experimental conditions are involved, so it is not clear why Nicholas and Thomas found the interaction to operate in the reverse direction for tomato in soil culture. The inference must

be/

be that a dependent variable factor was modified by the conditions of experiment. The high pH (6.5) of the soil used, together with the addition of cobalt in solution to soils which had received the heavy differential superphosphate dressings employed, suggests that precipitation of cobaltous phosphate took place (5, 147) or that positional availability of cobalt was operative.

A subsequent paper by Nicholas and Thomas (99) investigated the effects of nickel on the fertilizer and soil phosphate uptakes, again using tomato as the indicator plant. The soil and experimental techniques used were exactly as in the cobalt experiment just discussed. Once more it was not possible from the data provided to assess the effect of phosphorus treatment on the availability and uptake of nickel. In this case, in contrast to the findings for cobalt, increase in rate of phosphorus application had no effect on the intensity of the toxicity symptoms. Nickel was found to reduce the total iron content of the plants, although the iron status (as mg. per g.) was not related to the incidence of chlorosis, since the iron status actually increased with nickel treatment. The same criticism can be applied to this second part of the investigation regarding the possible loss of added nickel by precipitation as nickel phosphate which might not be readily available at the high soil pH.

Conclusions/

Conclusions

The factorial sand culture experiment has yielded information on the effects of variation in the supply of major nutrients on nickel toxicity. Two main effects emerged, concerning calcium and phosphorus. The remaining major elements, with the exception of nitrogen, played little part in influencing toxic symptoms. The phosphorus effect was particularly interesting, in view of its known effects on iron metabolism which had its implications in the interpretation of the nickel-iron relationship, as was pointed out earlier (see p.106).

ABSORPTION OF CHELATE METALS BY PLANTS

Note on the form of soil nickel in Whitecairns basin soil

The basin soils at Whitecairns are acid peaty loams with a high loss-on-ignition, which may reach 60%. Although the highest acetic-soluble nickel figures recorded by Vergnano were not for soils of this type, the toxic symptoms and uptake of nickel from them was generally greater than from the soils on the hill slopes. Clearly pH differences between the two soil types was the main factor involved since the pH of the basin soils was in the range 4.5 - 5.3 and that of the hill soils, 5.5 - 6.7. The amount of nickel obtained on leaching with neutral N-ammonium acetate has been shown to give good correlation with nickel uptake by oat plants. Here again, the mineral hill soils had "exchangeable" nickel contents quite as high and often higher than the basin soils.

The form in which the nickel occurred in these basin soils might play some part in affecting the amounts of nickel absorbed by the plants growing on them. It seemed reasonable to assume that the basin soils derived most of their nickel from that leached out from the hill slopes above, rather than from that set free by the weathering of their low mineral content or as a result of the high nickel content of the plant remains in their organic matter fraction. The peaty basin soils could then accumulate nickel from this source

which/

which would probably be absorbed mainly on the organic matter fraction of the soil rather than existing as free ionic nickel in the soil solution. Salmi (115) working in Finland, states that peat bogs act as accumulators of trace elements, and that there is often marked variation in the trace-element content from bog to bog. The content does not appear to depend on the botanical composition of the peat, but rather on its pH. The higher the pH of the peat, the lower the trace-element content and vice versa.

In this connection, the literature makes it clear that trace metals, mainly copper, added to soils which are peaty in character, are often held so firmly by the soil that normal extractants will not remove them. The inference is that metallo-organic complexes have been formed which have the effect of "fixing" the copper as far as plants are concerned. This type of behaviour led Heintze and Mann (45) to postulate that the unavailability of manganese in neutral and alkaline organic soils may be due to a mechanism of this sort. Further they found that only a very small part of the manganese added to such soils could be recovered by extraction with ammonium acetate. The recovery was markedly increased by the addition of low concentrations of copper, cadmium, nickel or zinc salts to the ammonium acetate extractant. This type of complex formation may well adversely affect the availability of soil manganese, but recent work with chelated iron in solution culture, which will be described later, suggests/

suggests that this form of iron is particularly suited to the plant's need, especially where translocation within the plant is concerned.

Interpretation of the results which follow now is difficult, not only because of the limited nature of the experiments performed but also because of the very complex system under consideration.

A bulk sample of Whitecairns basin soil was taken which had a pH of 4.6, and a loss-on-ignition of 52%. Duplicate 20 g. lots of air-dry soil were extracted overnight with the following extractants (a) water (b) 0.5 N acetic acid (c) aqueous 1% zinc sulphate (d) 1% zinc sulphate in 0.5 N acetic acid (e) 0.5% potassium sulphate. Agreement between duplicates was good and the mean values are given below.

Extractant	Nickel (ppm. in soil)
Water	7.2
Acetic acid	219
Zinc sulphate	294
$ZnSO_4$ in acetic acid	330
Potassium sulphate	17.0
blank due to $ZnSO_4$	7.0

The increase in nickel extracted by zinc sulphate, either in water or acetic acid, over that extracted by acetic acid alone was noteworthy. The low amount of nickel extracted by potassium sulphate suggests that the effect of zinc is unlikely to be due solely to a mass-action effect.

It/

It was concluded that the zinc was displacing nickel associated with the soil organic matter in a form not readily soluble in acetic acid. It was thought that this might be shown up by the extraction with (a) water or (b) acetic acid of soils which had been ignited to destroy organic matter. The low nickel values obtained - water soluble, 2 ppm.; acetic soluble, 16 ppm.; suggested that nickel had been converted into an oxide of low solubility.

Another method of destroying soil organic matter is by using successive portions of 20 vol. hydrogen peroxide added to the warmed soil suspension on a hot plate. It was realised that applied to a soil having a loss-on-ignition of 50% the process would take some time and that free organic acids might attack the mineral fraction of the soil and release nickel, which would give anomalous results. Four 20 g. samples of air-dried basin soil were peroxidised for 20 hours before all organic matter was destroyed. The soil suspension was then filtered, made up to 500 ml. and nickel determined in the filtrate. The mineral fraction which remained was extracted overnight with:- Samples 1 and 2, 0.5N acetic acid; Sample 3, aqueous zinc sulphate; Sample 4, zinc sulphate in acetic acid. The results are given below.

Sample No.	Nickel (ppm. in soil) Filtrate	Mineral Fraction	Total
1	730	87	817
2	760	80	840
3	739	34	773
4	758	73	831

The total nickel in this sample of basin soil was 1000 ppm. (estimated by semi-quantitative spectrographic method*).

The amount of nickel obtained by destruction of the soil organic matter followed by extraction of the residual mineral fraction approached the total nickel value in the soil obtained directly. From the figures available it is not possible to say whether the level of nickel found in the filtrate is derived solely from the organic matter or represents nickel associated with the mineral fraction in a relatively insoluble form, which was brought into solution by organic acids during the peroxide treatment. The lower value obtained on extracting the soil with zinc sulphate suggests that this latter may have been the case, but increase in concentration of the salt solution or use of a more active metal might have given an increase, which would have indicated that 1% zinc sulphate was not capable of freeing all the nickel associated with the organic matter. However, there seems no doubt that there/

* result supplied by Dr. D. J. Swaine.

there is some nickel in this peaty soil which is not extractable by 0.5 N acetic acid but which is brought into solution by zinc sulphate or zinc acetate.

Finally, a sample of mineral soil from the hill slopes at Whitecairns, having a pH of 6.3 and a loss-on-ignition of 26%, was extracted with (a) acetic acid (b) aqueous zinc sulphate and (c) zinc sulphate in acetic acid. The values (ppm. in soil) were 93, 140 and 150. The same trend as was found with the basin soil is seen here.

THE ABSORPTION OF CHELATE NICKEL BY OAT PLANTS

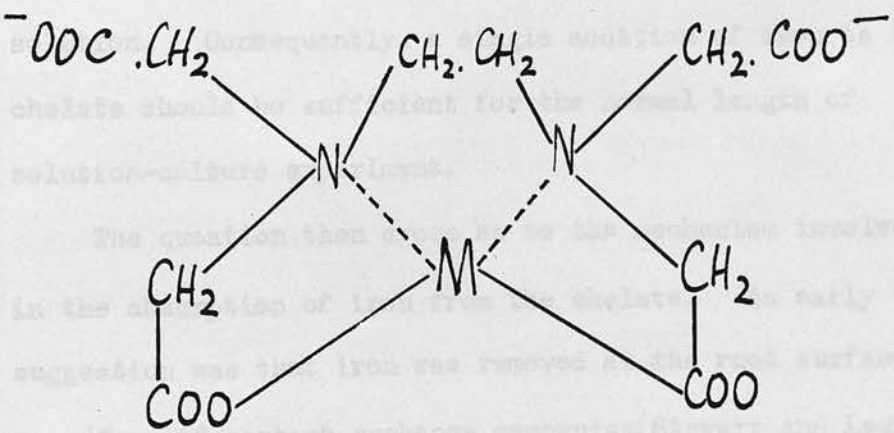
Chelating agents have the ability to form complex compounds with metal ions, in which the metal ions are held in soluble form with varying degrees of tenacity. When incorporated into a chelate compound, the metal ion loses its chemical identity and no longer reacts in the normal manner. Such agents e.g. oxines, carbamates, and certain classes of organic acid, find wide use in analytical chemistry where separations are involved. By varying pH, the non-specific character of aminopolycarboxylic acids such as EDTA may be lost so that certain metal ions are preferentially complexed although hydroxide and other precipitating anions exert an effect on the order of chelation. The chemical theory involved is complex, but, in general, for such acids the higher the valency and the higher the pH, the greater is the tendency for chelation to take place.

The presence of trace-metal contaminants in nutrient solutions always raises the question of their possible effects on the results obtained. In a paper published in 1950, Heck and Bailey (36) considered the use of chelating agents in nutrient solutions to complex such metals and render them unavailable to the plants. They investigated a number of the chelating agents which were then available and found that if supplied in the nutrient solution at a concentration high enough to complex the metals, severe

injury/

injury to the plants resulted. The authors suggested that metal ions were being removed from the roots by the chelating agents under these conditions. Lowered concentrations of the chelating agents which allowed normal plant growth were then found to be too low to complex the metals effectively. They concluded that the addition of chelating agents did not form an effective or feasible method of controlling trace-element contamination in nutrient solutions.

On the other hand, quite apart from their chelating activities, the compounds used by Heck and Bailey (which included Nitroso-R salt, chromatropic salt, 8-hydroxyquinoline, quinalizarin and oxine) were likely to be highly poisonous to plants at any but the lowest concentrations. This seemed an inherent drawback. In 1951, however, ethylenediamine tetra-acetic acid (EDTA) became available for use as a chelating agent. With di- and tri-valent metal ions it forms chelate complexes of the form shown below, where M is the metal ion.



Since chelate formation is associated with displacement of H^+, it follows that chelates should be more stable at high pH, and except for the most stable are extensively dissociated in strongly acid solutions. With EDTA, as the pH is reduced the stability of the chelate decreases. For most common metals, this loss of efficiency is not apparent until the pH drops to below 7.5, but varies from metal to metal. Thus at pH 5, calcium dissociated from the EDTA complex can be precipitated as oxalate, while copper, which is fully chelated at pH 3.5, is 50% dissociated at pH 2.

Jacobson (61) found the iron chelate a most satisfactory source of iron for plants grown in solution culture. The stability of the chelate was tested by allowing nutrient solutions of various pHs containing 5 ppm. iron (as the chelate), to stand in the dark for three months. No loss of iron occurred below pH 6. At pH 7, 15% of the iron had precipitated, and at pH 8, 30%. Even at pH 9, precipitation was not complete, about 10% of the iron remaining in solution. Consequently, a single addition of iron as the chelate should be sufficient for the normal length of solution-culture experiment.

The question then arose as to the mechanism involved in the absorption of iron from the chelate. An early suggestion was that iron was removed at the root surface by some form of contact exchange mechanism(Stewart and Leonard (124)). More recently, Wallace and North (138) in work dealing/

dealing with lime-induced chlorosis, concluded that there was evidence to show that the complete complex molecule was absorbed by the plant although there were indications that once in the plant the EDTA molecule becomes separated from the iron and may be broken down into simpler compounds and used by the plant. In the field of microbiology, Hutner et al. (58) were of the opinion that EDTA was not metabolised by micrörganisms. A further study of the problem was made by Weinstein, Robbins and Perkins (143) who employed a split-root technique to study the absorption and translocation of EDTA. Four basic treatments (pH 7.0) differing only in iron (as $FeSO_4$) or EDTA were used, which are sketched below.

	Normal culture	Split-root	
	0.5 ppm. Fe No EDTA	0.5 ppm. Fe	No Fe No EDTA
Roots:	poor	brown	brown
Tops:	chlorotic	chlorotic	

	Split-root		Normal culture
	0.5 ppm. Fe	5 ppm. EDTA	0.5 ppm. Fe 5 ppm. EDTA
Roots:	White, well developed.	Fe deficiency.	White, well developed.
Tops:	Green		Green.

The/

The plant used in these studies was sunflower and the authors conclude that EDTA is absorbed by the roots and that iron is, in some way, made available to the plants. In the left-hand culture iron absorbed at pH 7 is not in a form readily usable by the plant. Where EDTA was supplied it apparently migrated to the other half of the root system and to the top of the plant, chelating the iron and keeping it in a form available for metabolic use throughout the plant. Whether the iron chelate is transported without change to the leaves or whether the iron moves with some metabolised product of EDTA is not evident. Because of the high stability constant of Fe-EDTA (log K = 25) it would not be expected that this compound would readily give up its iron while still unchanged.

Amongst the chelates of the essential metals, Fe^{+++} is the most stable, and therefore not dissociated to any great extent in solution . Ni-EDTA has a lower stability constant (log K = 18.5) and although pH does not influence the order of stability constants, nickel is preferentially complexed at pH 6.5 before other divalent cations. Although in pure solution the presence of Ni^{++} would not be expected to affect the stability of the Fe-EDTA complex, conditions existing in a nutrient solution could possibly influence this and there might well be some exchange between the two to form Ni-EDTA and Fe^{+++}. In view of the fact that many of the experiments described/

described herein used Fe-EDTA in the presence of ionic nickel in the nutrient solution, it was reassuring to note that the results of the experiment described below, suggested that no exchange between the two took place in nutrient solution at pH 5.5.

It was decided to investigate the absorption of the nickel chelate when supplied to oat plants in sand-culture at a level known to produce relatively severe toxic symptoms (2.5 ppm. in the nutrient solution) if supplied as Ni^{++}. In forming the nickel chelate some excess of EDTA (1 mol. Ni to 1.5 mol. EDTA) was used so that no free nickel ions were left in solution. There were six treatments, each of two 9 in. clay pots, as follows:- a ferric citrate series (iron at 1.2 ppm.) with no nickel, ionic nickel, or nickel versenate. The same three treatments were used in a second series where the iron source used was Fe-EDTA.

The experiment lasted for 35 days. At the end of seven days the oat plants supplied with Ni^{++} had begun to show characteristic necrotic symptoms. No symptoms had appeared in the plants supplied with Ni-EDTA and the effect appeared to be independent of iron source. At harvest, the position as regards symptoms was unchanged. Fresh-tissue extracts were made of whole plants, and whole plants were dried for nickel and iron estimations. The material yielded by the two pots in each treatment was handled separately. No wide variation in major-nutrient composition or iron content had/

had been produced as a result of treatment but there were striking differences in the nickel contents as the table below shows.

TABLE 49

Absorption of nickel chelate of EDTA by oat plants

	Fresh Yield per pot (g.)	ppm. in fresh tissue extracts (mean values)				ppm. in dry matter		
		NO_3	P	K	Ca	Mg	Fe	Ni	Necrosis
Ferric citrate series									
Control	65.5	125	13.9	247	51.8	15.4	100	11.3	0
Ni^{++}	56.5	91	14.0	221	50.0	19.9	104	124.0	M
Ni-EDTA	72.9	113	13.8	251	34.0	15.7	91	9.7	0
Fe-EDTA series									
Control	67.6	129	16.0	248	35.2	17.9	107	8.4	0
Ni^{++}	57.7	119	15.9	231	39.0	17.1	105	100.0	M
Ni-EDTA	64.7	122	15.9	238	35.0	16.2	91	14.5	0

The lack of toxic symptoms where the nickel chelate was supplied had suggested that the Ni-EDTA had been absorbed but that the plants had been unable to break it down so freeing Ni^{++}. Experiments with rabbits have shown that levels of nickel greatly in excess of toxic doses as the free ion can be tolerated, provided that nickel is in the chelate form. From these results it was concluded that there are no natural complexing agents in the body which are capable of extracting nickel from the chelate.

Since/

Since the level of nickel in plants supplied with the nickel chelate did not vary significantly from that of the controls, whereas in plants receiving ionic nickel at the same concentration the level was more than ten times as great, the inference is that no absorption of the nickel chelate occurred. For the same reason, it would appear that the plants were unable to remove nickel from the chelate at the root surface, although this may well have been the mechanism operative in the case of iron. These results have been published in the form of a short note (23).

A second experiment (which lasted for 21 days) was conducted on the same lines as the first, but the amount of nickel chelate supplied in the nutrient solution was increased progressively to 25 ppm. The analytical results again showed that no absorption of nickel from the chelate had taken place and reference to the fresh weight yields in the table below indicates that some reduction of yield occurred in the Ni-EDTA treatments, due possibly to complexing of other essential cations by the excess EDTA present, although the levels of major nutrients in the fresh-tissue extracts of whole plants do not reflect these differences.

T A B L E/

TABLE 50

Effect of various levels of Ni-EDTA on growth of oat plants

Treatment	Fresh yield per pot (g.)	ppm. in fresh tissue extract				ppm. in dry matter		Necrosis
		P	K	Ca	Mg	Fe	Ni	
Control	40.9	18.2	237	50.4	19.6	84	4	0
2.5 ppm. Ni-EDTA	33.8	17.9	213	57.2	20.2	72	6	0
5.0 ppm. Ni-EDTA	37.6	20.2	234	52.0	20.2	71	4	0
10.0 ppm. Ni-EDTA	37.5	19.1	264	45.6	20.0	54	5	0
17.5 ppm. Ni-EDTA	33.6	18.7	259	60.0	17.8	81	5	0
25.0 ppm. Ni-EDTA	32.8	21.6	267	43.6	18.6	64	8	0

ABSORPTION OF CHELATE FORMS OF MANGANESE, COPPER, ZINC AND COBALT BY OAT PLANTS

In view of the above results obtained with chelated nickel which were in direct contrast to those for Fe-EDTA, it was considered worthwhile to study the absorption (or otherwise) of chelate forms of the essential trace-elements, copper, zinc, and manganese by oat plants. A treatment involving chelate cobalt was also included for comparison with nickel. As before, the basic oat solution was used with iron at normal level supplied as Fe-EDTA to the three pots in each treatment. It was decided to supply the metals (in ionic form) at a level which would be reflected in the plants on analysis and which would be much higher than that in control plants. It would thus be easier to interpret the results obtained from plants supplied with the same level of metal but in a chelate form. The levels at which the metals were supplied in the ionic form were copper, 15 ppm., zinc, 25 ppm., manganese, 150 ppm., and cobalt, 5 ppm. Once again, in preparing the chelates, an excess of EDTA was used so that free ions in solution were reduced to a minimum. It was found that when the volume of manganese chelate required was added to the nutrient solution its pH fell from 5.5 to just over 4.0. In order to maintain uniformity, the pH of all nine nutrient solutions was adjusted to pH 4.0. This course was adopted with some reluctance since growth at that pH would perhaps be adversely affected and secondly, the/

the dissociation of the chelates might be large enough to influence the results obtained.

These levels were expected to give some reduction in growth and possibly some iron-deficiency chlorosis when applied to sand-cultured oats. If the chelate metals (supplied at the same level) produced the same reduction in growth and their uptake by the plant was about the same as that found for ionic metals then it could be inferred that free absorption of the chelate had taken place.

The experiment ran for 30 days before harvesting. Heights and symptoms recorded during the experimental period indicated that the levels used were producing growth differences. Those noted at the end of 25 days will make this clear.

TABLE 51

	Average heights (cm.)		Chlorotic symptoms	
Treatment	Ionic	Complexed	Ionic	Complexed
Control	28 - 29	-	0	0
Cu	16 - 17	25 - 26	M	0
Zn	27 - 28	25 - 26	0	0
Mn	25 - 26	27	L	0
Co	27	28 - 29	M	0

Plants receiving [redacted] copper in the ionic form were most severely affected and ranged from pale green through yellow to dark brown, were stunted and appeared to be dying. The/

The complexed form of copper did not produce any symptoms although there was some slight reduction in growth. This was less evident at harvest when the heights (cm.) recorded were:-

	Ionic	Complexed
Control	32 - 33	-
Cu	19 - 21	32 - 33
Zn	31 - 32	32 - 33
Mn	31 - 32	31 - 32
Co	32 - 33	33

Only ionic copper has produced any marked reduction in growth. At harvest the yield per pot was assessed, first fresh, then dry, after drying overnight at 80°C. Major nutrients, iron and the metals concerned were determined in the dry matter of whole plants.

The yields of dry matter per treatment varied over fairly narrow limits, with the exception of these for ionic copper and complexed manganese, where yields were depressed; and complexed zinc where an increase in yield took place. The effects on major-element composition were not great; the reduction in total nitrogen due to Cu** agreed with the results of Hunter and Vergnano (57) who postulated that one of the toxic effects of copper was to induce nitrogen deficiency in oats. The increase in potassium content and reduction in calcium and magnesium contents, evident when each pair of treatments is compared, implies that this is

an/

TABLE 52

Analytical Results

Treatment	Mean yield of dry matter per pot (g.)	Percentage in drymatter					ppm. in dry matter				
		N	P	K	Ca	Mg	Fe	Cu	Zn	Mn	Co
Control	4.28	4.90	0.79	6.69	0.36	0.35	86	18.4	150	99	0.2
Cu^{++}	2.61	3.55	0.55	2.29	0.66	0.51	96	35.5	--	--	--
Cu-EDTA	4.33	4.95	0.77	8.45	0.38	0.24	94	33.7	--	--	--
Zn^{++}	4.47	4.23	0.73	5.63	0.48	0.37	95	--	1600	--	--
Zn-EDTA	4.89	4.88	0.72	7.39	0.37	0.27	80	--	206	--	--
Mn^{++}	4.22	4.44	0.71	5.81	0.31	0.32	87	--	--	5600	--
Mn-EDTA	3.88	4.77	0.69	7.74	0.26	0.28	82	--	--	530	--
Co^{++}	4.03	4.78	0.71	5.28	0.52	0.39	95	--	--	--	83
Co-EDTA	4.46	4.96	0.71	6.16	0.28	0.26	87	--	--	--	36

an effect due to EDTA since this is the only constant factor in the series; it being unlikely that each of the four metals would have the same effect on major-element content.

Conclusions

The results obtained from the experiments described in this section cast some light on the interesting differences which apparently exist in the availability of metals chelated by EDTA when supplied to oat plants. Chelate iron is absorbed as freely as ionic iron and is available for metabolic processes within the plant. On the other hand, the nickel and zinc chelates appear to behave similarly in that only traces of the metal are found in the plant. Occupying intermediate positions are cobalt and manganese, whose chelates appear to be absorbed to a certain extent although that of cobalt does not appear to be broken down in the plant. It is not possible to say whether the amount of manganese found in the plants was derived from the chelate after absorption, or whether it was normally-absorbed ionic manganese dissociated from the chelate.

The experiments carried out here are of a limited nature, and without much further detailed knowledge of the behaviour of the individual chelates in media as complex as a nutrient solution, it is impossible to ascribe possible reasons for the results obtained. The table below compares the absorption of each metal from chelate or ionic source and

lists/

lists this along with the relevant stability constants. The percentages are derived from the concentration (as ppm.) of each element found in the plant. When yield differences are taken into account and used to calculate the total content derived from each source, the percentages are not altered except in the case of copper where five times as much copper is found in the plants receiving Cu-EDTA. Of the metals considered, only iron is in the trivalent state and its chelate has the highest stability constant which is remarkable in view of its free absorption by plants.

TABLE 53

Behaviour of metal chelates in relation to their stability constants

Metal	log K	Relative absorption Ionic	Chelate	% absorption*
Fe^{+++}	25.0	equal amounts		100
Ni^{++}	18.5	91.6	6.1**	6.7
Cu^{++}	18.4	17.1	15.3	89
Zn^{++}	16.6	1450	56	3.8
Co^{++}	16.1	82.8	35.8	43
Mn^{++}	13.5	5501	431	7.8

Notes: * When compared on the basis of amount found in the plant (after deducting levels found in control plants) from ionic and chelate sources:-

$$\frac{\text{plant content from chelate source}}{\text{plant content from ionic source}} \times 100$$

** From results in Table 49. Other results from figures in Table 52.

The behaviour of the copper chelate is different from the rest in that analysis indicated that it is absorbed as freely as ionic copper, yet apparently remains non-toxic to the plants since no stunting or toxic symptoms appear. It therefore suggests that the chelate remains unchanged in the plant and thus prevents the usual metabolic upset associated with excess of ionic copper. Plants vary markedly in their tolerance to levels of elements in excess of their normal requirements. In a study of the flora of a copper-tailing region, Bateman and Wells (8) comment on the fact that while most of the vegetation was killed or dying, certain species notably wild rose, horsetail and some grasses, appeared unaffected although their tissues contained high concentrations of copper. A similar phenomenon was noticed in the Whitecairns area. The commonest weed found there is a deadnettle, which shows no toxic symptoms although its nickel content (up to 100 ppm. in the dry matter) is often higher than that of severely necrotic oat plants growing nearby.

While it is well known that different plant species require different levels of heavy metals before iron deficiency chlorosis is induced, it seems clear that some mechanism was operative in the plants mentioned above, which prevented copper or nickel from exerting their usual toxic effects. A mechanism involving complex or chelate formation could inactivate metals such as copper or nickel and certain organic acids present in plants can operate in this way, although they are much less powerful agents than say, EDTA.

GENERAL CONCLUSIONS

The results of the study of the basin soil at Whitecairns have shown that the beneficial effects brought about by liming are due to a reduction in the level of soil nickel extractable by N-ammonium acetate. The fact that the effect can be produced by both calcium and sodium carbonate indicates that pH change, rather than an increase in the soil calcium status, is responsible for the improvement in toxic symptoms. A series of laboratory experiments to test the recovery of nickel added to limed soils, has provided ample proof that this interpretation is correct.

The toxicity symptoms produced in oat plants by excess nickel are necrosis (a specific effect of nickel) and chlorosis brought about as a result of interference by the heavy metal with normal iron metabolism. No attempt has been made to ascertain the site of toxic action of nickel but it seems clear that the production of induced iron deficiency chlorosis is only one aspect of it.

In the field the absorption of nickel is greatest from soils of acid pH, decreasing as the soil pH is raised, which implies a precipitation or reduction in the solubility of the soil nickel. When the absorption of nickel at different pH values was determined in solution-culture, the opposite effect was found in that most nickel was absorbed in cultures of/

of pH 6 and above. The higher intensity of chlorosis seen at these pH values is not solely due to the reduced availability of iron under these conditions, although some reduction in uptake is noted in control plants as the pH of the nutrient solution is increased. From the results available it is not possible to say how this increased absorption is brought about, but it is possible that the pH change in the nutrient solution produced some change in the ionic form of nickel in the solution. These two sets of results serve to underline the inherent differences which exist between soils and the various culture media, and to stress the danger of using results obtained by the use of one technique to predict behaviour under a different set of conditions or in a different environment.

Nickel is found to reduce the concentration of iron in oat plants and examination of toxic tissue by means of auto-radiography has shown that iron is low in chlorotic tissue and absent in the necrotic areas. When toxic tissue from the Whitecairns area was analysed by chemical means it was found that the iron content of the necrotic areas was still quite appreciable although lower than that of whole toxic leaves or healthy leaves. The major-nutrient content did not vary widely between the three tissue types.

In the field it had been noted that toxic symptoms were most severe in young oat plants and that latterly, the youngest leaves which had been very chlorotic in the seedling/

seedling stage now unfolded normally and showed no chlorosis. The rate of absorption of nickel and iron by developing oat plants were followed when it was found that the concentration of iron, initially high in young plants, decreased slowly as the plants aged. In contrast to this, nickel in the plant increased rapidly with time until a maximum was reached at about 30 days. When the nickel-iron ratio in the plant is plotted against time a point of inflexion occurs at about 40 days which agrees well in point of time with the observed change in chlorotic symptoms.

Analysis of mature oat plants showed that iron and nickel both concentrated in the grain, more in young tissue than in old, and more in leaves than in stems. There were indications that nickel was more mobile than iron in the plant. This suggested a possible explanation for the absence of chlorosis in the last formed young leaves. Results which demonstrate the correlation between toxic symptoms and the nickel-iron ratio in the plant will be referred to later, but it is found that severe symptoms are associated with high values of the ratio. It follows then that in 40 to 50 day old plants, nutrients would already be moving into the developing grain and that since nickel is more mobile than iron, this would result in a lowered nickel-iron ratio in the young leaves then expanding, which might not then reach the critical level necessary for the production of chlorosis.

Variation/

Variation in the levels of major elements found in growing nickel-toxic plants in the above experiment were judged to be due to normal metabolic processes rather than a reflection of the nickel treatment. Similar trends were seen in healthy plants as they aged although the actual levels found often differed. Thus in the case of iron and phosphorus, the levels were lower in the nickel-toxic series, while for calcium the reverse was noted. Magnesium and potassium levels were similar in both types of plant.

Experiments were next undertaken to decide whether the nickel-iron antagonism occurred outside or inside the plant. It was known that a high-level iron supply prevented the appearance of nickel-toxicity symptoms although the level of nickel found in the plants was still appreciable. A series of "transfer" experiments was carried out to determine the factors affecting production of necrosis. It was found that plants cultured initially on a high iron supply when transferred to nickel-containing solutions became equally as necrotic as plants which had had an initial normal iron supply. Again, for plants raised on an optimum iron supply and then transferred to cultures containing nickel but increasing amounts of iron, the level of symptoms produced was governed by the amounts of nickel and iron in the nutrient solution, in fact by its nickel-iron ratio. A similar picture was obtained using tomato, which confirmed the external nature of the antagonism.

In/

In the experiment with tomato a split-root technique was adopted to give a type of culture intermediate between two normal cultures, one supplied with nickel, the other having no nickel. In the normal +Ni culture where the whole root system was in contact with nickel, a speedy poisoning of the root system took place, irrespective of level of iron supply. Where, however, only part of the root system was supplied with nickel (as in the split-root culture), although its further growth and secondary rootlet production was inhibited, the other half of the root system (without nickel), grew well and produced a plant of normal appearance containing only twice as much nickel as the control plants.

The very low nickel content of the split-root plants was difficult to explain especially in view of the fact that the "nickel" half of the root system contained fully as much nickel as the +Ni (normal) culture. A further complication was the fact that the very poor root system in the +Ni (normal) cultures had not prevented the absorption and translocation of major nutrients, although that of iron was somewhat reduced. Without further additional data or information, one is forced to conclude that the split-root plants preferentially utilized the "no-nickel" part of the system for the uptake of nutrients to the exclusion of the "nickel" portion, so reducing the amount of nickel translocated.

Once/

Once the external nature of the antagonism had been established, the effect of variation in the substrate nickel-iron ratio was studied to see whether it alone determined the intensity of toxic symptoms produced. The results indicated the importance of the ratio, but showed that the absolute amount of nickel present also had to be taken into consideration. Thus, while increase in the nickel-iron ratio produced symptoms of increased severity, for ratios of the same value, the symptoms produced depended to a large extent on the nickel level.

Root analyses of plants from these experiments showed many interesting features. Firstly, they showed that the high concentration of iron found in nickel-toxic roots was not primarily due to nickel, since healthy roots also had a high iron content which increased almost linearly with increase in level of iron supply. The movement of iron into the tops was practically constant irrespective of level found in the roots. As noted before, increasing iron had reduced the nickel content of tops, and root analysis now showed that it reduced nickel in the roots also.

Other effects which were noted included an increased uptake of calcium in the presence of nickel, and an apparent interference by nickel in the movement of potassium from roots to tops. This calcium effect has been discussed at some length under the section dealing with major nutrients, although it is impossible to explain how the effect comes about/

about from the data at present available.

When the effects produced in the field following fertilizer or lime applications were studied in detail in sand-culture experiments, the same trends were noted. In the factorial design, the effect of variation in the level of each major nutrient was determined and a quantitative estimate of the effect obtained by statistical analysis. As in the field, variation in calcium and phosphorus was found to produce the most marked beneficial or adverse effects on nickel toxicity, while nitrogen and magnesium gave lesser beneficial effects. Increase in potassium had little effect on toxicity symptoms or the uptake of nickel.

In the absence of pH changes, which account for differential uptakes of nickel from soils, the effects found here must be due to direct competition for absorption between the cations concerned. Primary differences between calcium, magnesium and potassium presumably depend on valency but those between calcium and magnesium must be affected to some extent by the increased absorption of calcium in the presence of nickel, although the indirect effect of magnesium on the nickel-iron ratio in the plant (by increasing the uptake of iron) will play some part in deciding the intensity of toxic symptoms produced.

In the case of the intensification of symptoms and increase in the uptake of nickel produced by increase in phosphorus supply, there appears to be some justification for

supposing that the effect occurs within the plant. The simplest hypothesis to account for this effect must take into account the effect of increased phosphorus supply on the absorption and translocation of iron, and more particularly, on the level of "active" iron found in the plant. A lowered iron status in the plant would result in increased nickel toxicity, as has been demonstrated in experiments to study the nickel-iron relationship.

The conflicting results obtained in the small experiment carried out to study the effect of molybdenum on nickel toxicity must be taken to indicate the delicate balance maintained by the plant between external factors such as pH, and the levels of trace elements in the plant. There seems little doubt that under certain conditions and perhaps only for certain plant types, molybdenum may exert a beneficial effect on the iron-deficiency chlorosis induced by excess of heavy metals. Further research may show that this effect is only obtained with certain heavy metals, thus emphasising the fact that all such metals probably do not produce iron deficiency in the plant by the same mechanism.

The assumption that much of the nickel in the peaty basin soil at Whitecairns is associated with the soil organic matter as a weak complex or chelate must be regarded as tentative. It is interesting to note that this nickel appears to be freely available to plants in contrast to the reduced/

reduced availability of copper added to similar peaty soils. A factor which should not be overlooked is the effect, known for many years, of humic acid of apparently facilitating the entry of nutrient ions into plants, and which now appears more logical in the light of recent results obtained with EDTA, which show that in the case of iron, the EDTA appears to aid in the movement of iron within the plant as well as having a beneficial effect on its absorption.

The non-absorption by oat plants of nickel from its chelate with EDTA is surprising in view of the ease with which plants can absorb the iron chelate, although the experiments with chelates of zinc, copper, manganese and cobalt indicate that a wide range of availability may be involved, depending ultimately on the stability of the chelate and possibly on the ease with which the metal concerned undergoes valency change. A more fundamental type of experimental approach would be required before the full picture could be built up and reasons given to account for the observed differences in availability of the chelates.

The same holds true for further work on nickel toxicity, particularly in relation to iron metabolism. Sand and solution-culture studies serve to show the magnitude and direction of the factors involved, but a final assessment can only be made after a fuller picture of how nickel affects cell metabolism has been worked out.

SUMMARY

The occurrence of nickel-toxic serpentine soils at Whitecairns, Aberdeenshire, led to an investigation of the effects of excess nickel on plants, together with a study of the soil factors affecting nickel absorption. The oat plant was selected as a suitable indicator plant. The results are summarised as follows:-

1. Nickel, in common with other heavy metals, induces iron deficiency when supplied in excess to plants, which vary in their tolerance to the toxicity. In oat plants, which are particularly sensitive, white stripes or bands of necrotic tissue are produced in addition to the chlorosis associated with iron deficiency. The chlorosis responds to painting or spraying with solutions of iron salts whereas the necrosis does not.

2. Examination of nickel-toxic oat tissue by means of autoradiographs has shown that iron is low in the chlorotic areas and absent in the necrotic patches. Analysis of oat leaves from plants growing on the toxic serpentine soils confirmed these findings although some iron was still detected in the necrotic tissue, where in general, nutrients were at a lower level than in the rest of the leaf, suggesting a migration of nutrients out of this dying tissue.

3. The absorption of nickel by young oat plants increases rapidly from germination for about 30 days when the concentration in/

in the tissues reaches a maximum and thereafter starts to decrease slowly. The concentration of iron, on the other hand, starts at a high level in the young plants and falls only slightly with age. This change in rate of absorption of nickel is accompanied by change in the (visual) toxicity symptoms. Up till 50 days young leaves unfold chlorotic whereas after that time they emerge a normal green colour. Basal and intermediate leaves are necrotic but seldom chlorotic. When the nickel-iron ratio in the plant is plotted against time, the maximum point on the curve coincides in point of time with the observed change in symptoms. The changes produced by nickel in the uptake of major nutrients were also followed. The concentrations of iron and phosphorus were always lower in nickel-toxic than in control plants, whereas that of calcium was always higher. Magnesium and potassium content were not dissimilar in both types of plant at the same stage of growth. The change in concentration of the major nutrients in the growing plant were probably not a reflection of nickel treatment, but rather of normal metabolic processes in the plant, associated with the translocation of nutrients to the developing grain. In mature oat plants, nickel concentrates in the grain, more in young tissue than in old, and more in leaves than in stems.

4. The visual symptoms produced in plants by excess nickel result from an upset in iron metabolism. Experiments were therefore/

therefore designed to examine this antagonism. It was found that increase in iron supply reduced toxic symptoms and the uptake of nickel, although plants showing no visual symptoms still contained appreciable amounts of nickel. It was confirmed that a reciprocal relationship exists between the nickel and iron contents of the plant; the nickel content is reduced by high concentrations of iron in the nutrient solution and the iron content by nickel, the former being the more pronounced effect. The reduction in the degree of necrosis is related to a reduced nickel content in the plant while that of chlorosis is related to the nickel-iron ratio in the plants. The absolute amounts of iron and nickel in the substrate rather than the iron status of the plant determine the degree of symptoms and uptake of nickel, although the ratio of nickel to iron in the substrate also plays a part in deciding how severely plants will be affected. Narrow ratios are associated with severe symptoms; while for the same nickel-iron ratio, symptoms increase in severity as nickel in the nutrient solution increases. The degree of symptoms and the nickel-iron ratio in the plant are connected linearly. The external nature of the nickel-iron antagonism was confirmed in a split-root experiment using tomato as indicator plant.

5. The absorption of nickel by oat plants was studied in solution culture and is found to increase with increasing pH; but this increased absorption of nickel is not simply due to a decreased availability of iron as a result of the high pH of the/

the cultures. The same degree of necrosis is found over pH range 4 - 7 but chlorosis is more severe above pH 6. In the field, increase in soil pH brought about by liming, results in lowered absorption of nickel and reduced toxic symptoms, suggesting a reduction in the availability of the soil nickel. The beneficial effect is due solely to the pH change involved and is not dependent on an increased calcium status, since sodium carbonate will produce the same result. It is found that the amount of soil nickel extractable by N-ammonium acetate falls with increase in soil pH. Laboratory studies on the recovery of nickel added to soils of varying pH (produced by the addition of either calcium or sodium carbonate) gave the same result. In a series of pot-culture experiments using toxic soils limed with either calcium or sodium carbonate to give a range of soil pHs, the uptake of nickel by the plants and the degree of symptoms observed agreed well with the "exchangeable" nickel removed from the soils after the experiment by leaching with ammonium acetate.

6. Field observations indicated that liming reduced the toxic symptoms in oats while applications of phosphorus fertilizers made symptoms worse. Nitrogen also improved growth while potash and magnesium applications had little effect. Sand-culture experiments have been used to examine the effect of variation in major nutrient supply on nickel toxicity symptoms and uptake of nickel. Increase in the concentration of cations in the nutrient solution was found to affect/

affect the toxic symptoms and nickel uptake markedly. The largest beneficial effect was obtained with calcium or magnesium and least with potash. In the absence of pH changes, which accounted for differences in uptake of nickel from soils, the effects found here must be due to direct competition for absorption between the cations concerned. Increase in rate of phosphorus supply was still found to increase symptom degree and uptake of nickel. The simplest hypothesis to account for this effect would involve reduction in the level of iron absorbed and translocated, and as has been pointed out earlier, a lowered iron status in the plant would result in increased nickel toxicity. Nickel was found to increase the concentration of calcium in the tissues but from the available data no explanation for this phenomenon can be offered.

7. Molybdenum has been found by some workers to cure the iron deficiency chlorosis induced by certain heavy metals. No definite indication of any such beneficial effect was noted when molybdenum was supplied to nickel-toxic oat plants.

8. The soils from the most severely affected area at Whitecairns are characterised and compared with several uncultivated serpentine soils from other parts of Aberdeenshire. The mineral soils from Whitecairns are found to be generally higher in nickel than other Aberdeenshire serpentine soils. The Whitecairns soil which produced the most severe cases of nickel/

nickel toxicity, is a very peaty soil occurring in a low-lying basin at the foot of a slope of mineral serpentine soil. Nickel leached out of this mineral soil accumulates and is firmly retained by the peaty soil. There is some evidence that the nickel in this soil is held on the organic matter by some form of loose chelate bonding from which it can be displaced by extraction with a 1% solution of zinc sulphate although not by dilute acetic acid.

9. Two forms of iron were used in these plant culture studies; ferric citrate and a chelate form employing ethylene-diamine tetra-acetic acid (EDTA). Both appeared equally good sources of iron and when certain aspects of the work were repeated using the chelate form identical results were obtained. There was no indication of any exchange between ionic nickel present in the nutrient solution and the chelate form of iron. Further evidence of this was supplied indirectly when it was found that nickel complexed by EDTA was unavailable to oat plants. The level of chelated nickel supplied was increased to 25 ppm. without any nickel being absorbed by the plants. This level of nickel would be very toxic to oats if supplied in ionic form. The availability of other heavy metal chelates also showed interesting differences.

10. An existing colorimetric method for the estimation of nickel was successfully adapted to permit of its use on plant and soil extracts.

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A P P E N D I X

TABLE 1

Composition of stock solutions

Major nutrient solutions

$NaNO_3$	250	g. per litre.
$Na_2HPO_4 \cdot 12H_2O$	50	
K_2SO_4	90	
$Ca(NO_3)_2 \cdot 4H_2O$	430	
$CaCl_2$	453	
$MgSO_4 \cdot 7H_2O$	250	

Trace-element solutions

Ferric citrate trace solution

Ferric citrate	6.40	g. per litre.
$MnSO_4 \cdot 5H_2O$	0.98	
$CuSO_4 \cdot 5H_2O$	0.04	
H_3BO_3	0.35	

Use 10 ml. per 10 litres of nutrient solution.

Table 1 continued

Fe-EDTA trace solution

This solution contains the same concentration of iron as the ferric citrate trace solution, and is prepared as follows:

26.1 g. ethylenediamine tetra-acetic acid dissolved in 268 ml. N. KOH, 24.9 g. $FeSO_4.7H_2O$ added, dissolved, diluted to 1 litre and aerated overnight. Use 2.4 ml. per 10 litres of nutrient solution. Manganese, copper and boron are added separately from a solution containing the same amounts of the appropriate salts as used in the ferric citrate trace solution.

Ni (1000 ppm.)	$NiSO_4.6H_2O$	4.63	g. per litre.
Co (1000 ppm.)	$CoSO_4.7H_2O$	4.76	
Cu (1000 ppm.)	$CuSO_4.5H_2O$	3.93	
Zn (1000 ppm.)	$ZnSO_4.7H_2O$	4.41	
Mn (1000 ppm.)	$MnSO_4.4H_2O$	4.65	
Mo (1000 ppm.)	$Na_2MoO_4.2H_2O$	2.52	

TABLE 2

Effect of age of oat plants on absorption of nickel and iron

Treat. No.	Age of plants (days)	Dry weight (g.) per 100 plants	Percentage in dry matter					ppm. in dry matter		Toxic symptoms*		Ni/Fe ratio
			N	P	K	Ca	Mg	Fe	Ni	Necrosis	Chlorosis	
823	+ 4	0.459	4.78	0.688	1.59	0.450	0.283	85	27	0	0	0.32
824	+ 9	1.14	4.40	0.538	2.38	0.583	0.363	73	30	L	0	0.41
825	+14	2.31	4.63	0.565	3.44	0.628	0.403	71	56	L	L	0.79
826	+18	3.07	5.13	0.523	4.02	0.660	0.420	79	68	L	L	0.86
827	+22	3.96	5.36	0.550	4.54	0.650	0.378	65	83	L	L	1.28
828	+26	5.64	4.84	0.538	4.76	0.440	0.300	78	100	L	L	1.28
829	+29	8.96	4.20	0.495	4.24	0.485	0.358	66	98	L	M	1.48
830	+33	12.24	3.68	0.373	3.96	0.340	0.283	72	83	L	M	1.15
831	+34	12.25	4.24	0.390	3.74	0.490	0.333	64	94	L+	M	1.47
832	+37	21.71	3.68	0.330	3.36	0.408	0.310	51	85	L+	M	1.67
833	+37	22.65	4.04	0.397	4.02	0.485	0.328	68	95	L	M+	1.40
834	+39	24.08	3.27	0.248	3.03	0.320	0.223	38	74	L	H	1.95
835	+41	24.20	2.64	0.261	3.02	0.440	0.283	59	89	L	M	1.51
836	+42	31.76	3.14	0.262	3.03	0.500	0.305	48	87	L+	H	1.81
837	+44	33.71	2.61	0.239	2.69	0.385	0.267	51	78	L	M	1.53
838	+45	31.07	2.82	0.253	3.07	0.468	0.264	63	82	L+	H	1.30
839	+48	42.11	2.49	0.215	2.47	0.360	0.253	73	73	L	L	1.00
840	+49	60.76	2.46	0.196	2.38	0.500	0.243	52	78	L+	L+	1.50
841	+51	47.99	2.48	0.238	2.64	0.463	0.290	57	75	L	0	1.32
842	+52**	48.76	2.13	0.177	2.27	0.413	0.206	48	73	M	0	1.52
843	+55	44.54	2.10	0.206	2.20	0.298	0.206	65	68	L	0	1.05
844	+56	82.53	1.91	0.171	2.07	0.395	0.238	40	68	M	0	1.70
845	+58	57.02	2.23	0.200	2.25	0.413	0.243	64	78	L+	0	1.22
846	+59	75.06	2.21	0.195	2.20	0.440	0.249	51	97	M	L-	1.90
847	+62	66.68	2.15	0.226	2.40	0.424	0.263	75	64	L+	0	0.85
848	+63	88.61	1.76	0.168	1.89	0.408	0.218	62	65	M	L	1.05
849	+64	67.43	2.15	0.234	2.29	0.413	0.276	78	68	L+	0	0.87
850	+66	111.3	1.74	0.157	1.63	0.441	0.217	59	64	M	0	1.08
851	+69	74.17	2.24	0.220	1.98	0.550	0.295	93	76	M	0	0.82
852	+73	62.27	2.39	0.206	2.09	0.473	0.290	79	75	M	0	0.95

* On scale H (high), M (medium), L (low), 0 (healthy); further differentiation by means of + or - signs.
** Grain emerged at this stage of growth.

TABLE 3

Design of factorial experiment to study effect of major nutrients on nickel toxicity

1 22222 Ni	2 11122	3 12222	4 22112	5 11221 Ni	6 12121 Ni	7 21111 Ni	8 11212 Ni	9 12211	10 11111	11 22121	12 21122 Ni	13 21212	14 22211 Ni	15 12112 Ni	16 21221
17 12121	18 12222 Ni	19 12112	20 11122 Ni	21 22211	22 11221	23 22222	24 21111	25 21212 Ni	26 22112 Ni	27 21221 Ni	28 12211 Ni	29 11111 Ni	30 21122	31 11212	32 22121 Ni
33 12111	34 11222	35 12122	36 22111 Ni	37 21211 Ni	38 21112	39 11211	40 11121 Ni	41 22212	42 12221 Ni	43 11112 Ni	44 22221	45 21121	46 12212 Ni	47 21222 Ni	48 22122 Ni
49 12122 Ni	50 22221 Ni	51 11211 Ni	52 12221	53 11112	54 22212 Ni	55 21222	56 21211	57 22111	58 12111 Ni	59 21121 Ni	60 12212	61 11121	62 21112 Ni	63 11222 Ni	64 22122

Note: The five-figure groups are a short method of indicating the different levels of N, P, K, Ca and Mg supplied. E.G. 22222 implies $N_2P_2K_2Ca_2Mg_2$. Nickel treatments indicated by chemical symbol for nickel under appropriate treatments.

TABLE 4

Yield and analytical data for factorial experiment

Treatment**	Number in design	Yield of dry matter (g.)	Percentage in dry-matter of whole plants N	P	K	Ca	Mg	ppm. in dry-matter of whole plants Fe	Ni	Toxic* Symptoms
Nil	10	82.5	3.99	0.401	4.08	0.360	0.238	26.8	6.9	0
N	24	113.1	4.15	0.350	2.95	0.260	0.199	23.8	8.8	0
P	33	98.4	3.75	1.045	3.20	0.330	0.249	31.5	7.5	0
NP	57	134.1	3.85	0.908	2.40	0.260	0.190	20.3	4.4	0
K	39	89.0	3.78	0.403	6.13	0.270	0.182	21.3	3.8	0
NK	56	116.0	3.84	0.306	5.33	0.230	0.168	22.0	4.7	0
PK	9	87.6	3.75	0.997	5.65	0.250	0.201	28.8	10.6	0
NPK	21	125.0	3.92	0.770	5.25	0.230	0.190	25.0	7.5	0
Ca	61	91.3	3.93	0.377	3.93	0.420	0.182	23.5	4.4	0
NCa	45	84.2	3.69	0.363	3.45	0.360	0.171	21.0	5.0	0
PCa	17	88.7	3.67	0.927	3.95	0.460	0.210	26.3	7.5	0
NPCa	11	108.6	3.99	0.942	2.93	0.380	0.211	26.3	7.8	0
KCa	22	78.5	3.83	0.550	6.00	0.390	0.179	15.8	7.5	0
NKCa	16	93.0	3.90	0.323	5.33	0.330	0.140	26.8	6.9	0
PKCa	52	103.2	3.38	0.785	5.65	0.350	0.182	21.3	9.7	0
NPKCa	44	119.4	3.56	0.770	5.00	0.340	0.164	21.0	5.0	0
Mg	53	83.1	3.85	0.339	3.73	0.220	0.357	15.3	4.4	0
NMg	38	99.5	3.74	0.306	2.88	0.190	0.302	22.0	5.6	0
PMg	19	92.1	3.66	1.028	3.55	0.255	0.381	26.8	10.6	0
NPMg	4	101.8	4.27	0.889	2.48	0.180	0.350	25.0	7.5	0
KMg	31	84.5	3.28	0.398	6.40	0.180	0.260	23.8	6.3	0
NKMg	13	85.1	3.87	0.327	5.25	0.170	0.289	29.5	7.8	0
PKMg	60	107.7	3.56	0.855	5.65	0.170	0.306	20.3	6.9	0
NPKMg	41	115.7	3.48	0.750	5.00	0.160	0.291	20.0	7.5	0
CaMg	2	83.3	3.86	0.368	4.08	0.360	0.306	31.5	7.5	0
NCaMg	30	95.8	3.93	0.312	3.08	0.290	0.263	26.8	10.3	0
PCaMg	35	89.3	3.81	0.994	3.65	0.330	0.306	28.5	9.7	0
NPCaMg	64	117.1	4.04	0.885	2.55	0.290	0.319	18.5	5.0	0
KCaMg	34	89.6	3.81	0.420	5.93	0.320	0.256	28.8	7.5	0
NKCaMg	55	92.1	3.92	0.315	5.08	0.280	0.206	14.5	6.6	0
PKCaMg	3	87.0	3.53	0.911	6.08	0.320	0.273	28.3	13.1	0
NPKCaMg	23	103.5	3.71	0.788	5.33	0.300	0.293	22.8	6.9	0
Ni	29	63.8	3.81	0.397	3.25	0.520	0.210	15.3	90.7	9 - 2
NNi	7	84.2	4.38	0.350	2.88	0.360	0.214	26.0	80.0	4 - 2
PNi	58	72.3	3.83	1.031	3.73	0.540	0.287	12.8	99.0	10 - 5
NPNi	36	101.8	3.90	0.908	2.53	0.360	0.168	18.8	67.5	3 - 3
KNi	51	72.3	3.75	0.420	5.85	0.310	0.195	13.0	67.5	8 - 4
NKNi	37	103.8	3.89	0.350	4.68	0.290	0.153	17.8	67.7	3 - 1
PKNi	28	82.2	3.82	0.935	5.48	0.350	0.234	21.0	86.9	6 - 4
NPKNi	14	109.7	3.84	0.860	4.68	0.290	0.201	20.8	70.0	1 - 3
CaNi	40	74.8	3.63	0.377	3.60	0.600	0.144	18.5	80.3	3 - 3
NCaNi	59	90.4	3.68	0.305	2.80	0.450	0.144	16.3	70.8	<1 - 1
PCaNi	6	76.3	3.44	0.997	3.45	0.665	0.206	21.0	92.5	7 - 3
NPCaNi	32	96.1	4.01	0.908	2.68	0.530	0.186	14.3	73.1	3 - 3
KCaNi	5	71.2	3.61	0.356	5.73	0.570	0.171	25.5	92.3	4 - 2
NKCaNi	27	91.6	3.80	0.314	5.00	0.420	0.118	11.8	60.7	<1 - 1
PKCaNi	42	87.0	3.35	0.860	5.33	0.500	0.182	16.8	92.8	4 - 3
NPKCaNi	50	112.6	3.95	0.722	4.85	0.450	0.112	15.0	63.8	2 - 4
MgNi	43	67.2	3.53	0.354	4.00	0.288	0.409	20.3	80.0	4 - 3
NMgNi	62	98.7	3.93	0.336	3.20	0.230	0.298	16.3	72.8	2 - 1
PMgNi	15	67.2	3.62	0.986	3.98	0.300	0.508	23.3	93.8	6 - 4
NPMgNi	26	88.2	4.00	0.885	2.60	0.220	0.413	18.5	70.3	3 - 2
KMgNi	8	73.1	3.61	0.375	5.73	0.200	0.328	29.5	86.3	1 - 2
NKMgNi	25	82.5	3.54	0.293	5.00	0.180	0.293	11.3	61.0	1 - 1
PKMgNi	46	81.9	3.37	0.833	5.33	0.200	0.341	19.0	79.8	4 - 3
NPKMgNi	54	111.4	3.98	0.724	4.68	0.170	0.302	16.0	49.4	1 - 3
CaMgNi	20	79.1	3.53	0.360	3.93	0.450	0.324	26.8	83.1	1 - 2
NCaMgNi	12	85.9	3.88	0.312	3.05	0.340	0.280	24.8	70.6	<1 - 1
PCaMgNi	49	88.5	3.52	0.810	3.33	0.380	0.326	26.5	74.4	3 - 4
NPCaMgNi	48	90.2	3.79	0.855	2.68	0.340	0.333	19.8	76.3	1 - >3
KCaMgNi	63	90.7	3.54	0.350	5.85	0.360	0.276	19.8	82.5	1 - 1
NKCaMgNi	47	90.7	3.38	0.315	4.93	0.300	0.245	21.3	70.3	0 - >1
PKCaMgNi	18	79.1	3.58	0.881	5.85	0.400	0.319	22.0	87.5	3 - 4
NPKCaMgNi	1	93.6	3.80	0.803	5.08	0.380	0.311	25.7	77.5	1 - 3

** The level of treatment applied is indicated by the presence or absence of the chemical symbol for the nutrient concerned. If present, the nutrient was supplied at level 2 (high) if absent, at level 1 (normal). "Nil" implies that all nutrients were supplied at normal level in the absence of nickel. "PKCa" means $N_1P_2K_2Ca_2Mg_1Ni_0$.

* Toxicity symptoms were scored on scale 0 (healthy) - 10 (very severe) for necrosis, and 0 (healthy) - 5 (very high) for chlorosis. Necrosis scores are placed first.